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VITAMIN B REQUIREMENTS FOR SUCCESSFUL REPRODUCTION AND REARING OF THE YOUNG

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Increasing the various constituents in a synthetic ration satisfactory for normal growth has never caused satisfactory reproduction and rearing of the young compared to that obtained by feeding mixtures of natural foodstuffs. Evans and associates (1922), (1923), Sure (1924) and Mattill (1926) have produced experimental evidence to show that in addition to the dietary requirements for growth, another factor is necessary for reproduction. Nelson (1923) and Hogan (1924) have obtained animals in the third and fourth generation respectively by increasing the yeast content of the purified rations. However, the mortality of the young was high and their rations contained butter fat which, according to Evans (1923) and Mattill (1926), contains this reproductive factor. Any improvement in reproduction and lactation observed through increasing the per cent of yeast in the diet would naturally cause one to surmise that the increased intake of vitamin B was the cause of this increased production. Addition of yeast to a ration increases the content of certain other nutrients and the writer is of the opinion that these nutrients are responsible to a larger degree for the success, providing the original concentration of vitamin B was adequate for normal growth. Contrary to the opinion of Sure (1924), and Guest (1926), evidence will be presented in this paper to show that the concentration of vitamin B in a ration satisfactory for reproduction and lactation need not be greatly increased, if at all, over that in a ration satisfactory for growth.

Supplementing a synthetic ration with 5 or 10 per cent wheat embryo or an alcoholic extract of wheat embryo causes reproduction at normal intervals (Miller, 1924). Evans (1925) has demonstrated that the oil from wheat embryo is an excellent source of the reproductive vitamin.

The rearing of young, however, on this ration is not satisfactory. A high mortality occurs and especially at the weaning stage, strongly indicating the presence of some toxic principle. McCollum (1916) observed this in some of his earlier work. Results will show in this paper that the toxicity may be destroyed by heating the wheat embryo in an autoclave.

Purified food rations supplemented with corn or wheat embryo as the source of vitamin B are inadequate for growth if the corn and embryo have been autoclaved at fifteen pounds pressure for two and one-half hours.

TABLE 1

Composition of diets

DIET NUMBER	CASEIN	SALTS	AUTOCLAVED WHEAT EMBRYO	COD LIVER OIL	YEAST	DEXTRIN	WHEAT EMBRYO
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
1	18	4	10	2	3 (no. 1)	63	
2	18	4	10	2	1 (no. 1)	65	
3	18	4	10	2	3 (no. 2)	63	
4	18	4	10	2		66	
5	18	4		2		66	10
6	18	4		2	3 (no. 1)	71*	

* Agar, 2 per cent.

TABLE 2

Reproduction records

DIET NUMBER	NUMBER OF MALES	NUMBER OF FEMALES	NUMBER OF LITTERS	TOTAL YOUNG	YOUNG ALLOWED TO DEVELOP	YOUNG REARED
1	4	6	12	104	73	67
1*	4	5	5	33	30	30
2	2	3	0	0	0	0
3	2	3	0	0	0	0
4	5	3	0	0	0	0
5	2	3	3	18	18	4
6	2	3	2	11	11	5

* Second generation.

This is undoubtedly due to the destruction of vitamin B by heat, previously observed by Nitzescu (1923) and Sherman (1923). If small quantities of yeast are added to the above rations normal growth not only occurs but also satisfactory reproduction and rearing of the young. Employment of the above ration therefore affords an opportunity to study the vitamin B requirements during gestation and lactation as they may be controlled at will through definite additions of yeast or other vitamin B concentrates to a ration otherwise satisfactory.

EXPERIMENTAL. The compositions of the rations used are given in table 1. Dried samples of yeast from two different sources were used and they are referred to here as no. 1 and no. 2. The casein was a commercial casein which had been washed with distilled water slightly acidified with acetic acid. The quality of the salt mixture has been described in a previous article (Miller, 1924). Dextrin was prepared by autoclaving starch with 0.5 per cent citric acid for three hours at 15.0 pounds pressure. In autoclaving the wheat embryo it was placed in a crock in layers not over four inches thick and subjected to a steam pressure of 15 pounds for two and one-half hours.

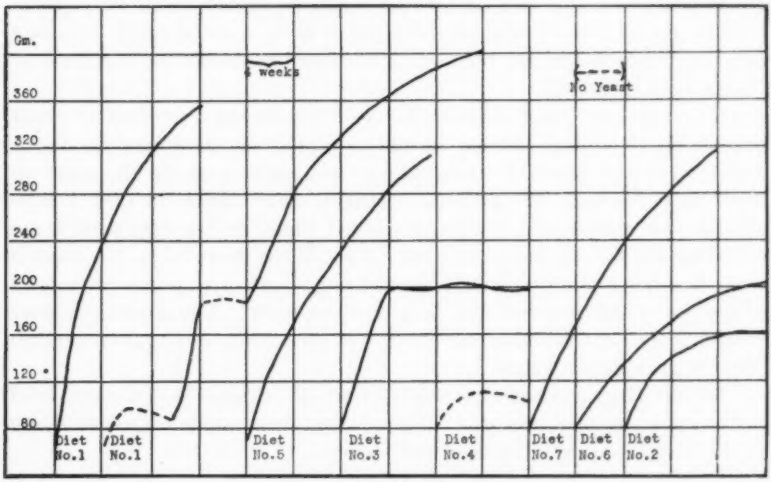

Fig. 1.

The animals used were taken from the stock colony at 29 to 32 days of age. Feed was always present in the cup, but no record of food consumption per individual animal was kept. The weights of feed consumed by the individual groups were recorded and as each original group contained two males and three females the average food consumption per animal could be satisfactorily calculated. Shavings was used as a litter for all the experimental groups.

DISCUSSION. Data here presented show that with a diet adequate in all respects except as to vitamin B, satisfactory growth, reproduction and rearing of the young are obtained by supplementing with 3 per cent yeast. Diet 4 containing no yeast was a failure in supporting growth, addition of 1 per cent yeast or diet 2 improved conditions, but did not support normal growth as shown in the curves of figure 1. These curves

are representative of the males in groups receiving the corresponding rations. Diet 3 containing 3 per cent of another variety of yeast was not satisfactory for growth, but previous work in this laboratory had demonstrated that this yeast, 2, was only about 40 per cent as potent in vitamin B as yeast 1.

These marked differences in animal production caused by a slight variation in the yeast content support the statement previously mentioned by the writer that the percentage of vitamin B in a ration satisfactory for the complete reproductive cycle need not be much greater, if at all, than that in a ration satisfactory for normal growth. It is probable that exact differences in vitamin B requirements could be satisfactorily worked out by this method of feeding where vitamin B in a satisfactory breeding ration could be destroyed by heat and its level controlled by certain concentrates of vitamin B.

The growth curves of animals on diet 6 containing 3 per cent of yeast but no wheat embryo did not ascend as rapidly as the animal on diet 1. The food consumption of these animals was approximately the same up until they reached 200 grams in weight when those on diet 1 consumed more per animal. The autoclaved wheat embryo apparently accelerated growth in some manner. Two litters were born to animals receiving diet 6 and one female weaned her young. At thirty-three days of age the young averaged only 33 grams in weight. Young from mothers receiving the autoclaved wheat embryo in the ration always average from 50 to 60 grams at this age.

The fact that the toxic principle in wheat embryo is destroyed is of importance not only in a study like this, but in a more extensive study of the nutritive properties of wheat embryo.

SUMMARY

The percentage of vitamin B in a ration satisfactory for successful gestation and lactation need not be greater than in a ration supporting normal growth.

The toxic principle in wheat embryo may be destroyed by autoclaving the embryo at fifteen pounds pressure for two and one-half hours.

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BLOOD REGENERATION IN SEVERE ANEMIA[1]

V. Influence of Striated and Smooth Muscle Feeding

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In long-continued severe anemia in healthy dogs we are able to measure with considerable accuracy the potential hemoglobin-forming capacity of any single diet factor or combination of diet factors. In the first papers of this series we outlined carefully all experimental procedures and methods which need not be reviewed here.

We may recall that it is important to maintain a *constant maximal stimulus* for new hemoglobin production and this is done by continuing the anemia by suitable bleedings at a level of 40 to 50 per cent hemoglobin. The normal dog under exactly similar conditions will show a level of hemoglobin between 130 and 150 per cent hemoglobin using 13.8 grams hemoglobin per 100 cc. as the 100 per cent standard,—therefore our experimental anemia level is one-third normal and gives a sustained maximal stimulus for hemoglobin production. In spite of this anemia these dogs are in every way normal in activity, appearance and appetite. Every effort is made to keep these dogs healthy by means of isolation, room ventilation and heat regulation, exercise periods, etc. We have kept a colony of dogs under these strict experimental conditions for a maximum of 15 months and so far we have observed no difference in the type of blood cell and hemoglobin regeneration in such dogs during the second month of the anemia as compared with the 15th month. We expect to follow some of these anemic animals through their entire life cycle with the hope that the bone marrow may eventually become exhausted and disturbed in some fashion so as to produce abnormal red cells.

It is important to bear in mind that our figures for hemoglobin production week by week do not include the unknown *maintenance factor*. By this term we mean the hemoglobin which must be produced week by week

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to make up the wear and tear or physiological wastage of red cells and hemoglobin in the circulation. Unfortunately the duration of the life cycle of the red cell in the normal dog is not known nor can we even make a respectable guess as to this most important and fundamental unit measure. Until we know this life period of the red cell we cannot measure total hemoglobin produced in absolute values but one feels considerable confidence that under these experimental conditions this *maintenance factor* is reasonably constant. The various facts known about the red cell life history are discussed in the first paper (Whipple and Robscheit-Robbins, 1925a) of this series.

The tables given below illustrate the favorable reaction which follows a diet of cooked *striated muscle* (heart or skeletal). A notable reaction to feeding chicken *smooth muscle* (gizzard) is recorded but the reaction to a diet of cooked stomach of beef animals or uterus of the pig is not remarkable.

METHOD. The technique of the various experimental procedures has been described in detail in the first paper of this series (Whipple and Robscheit-Robbins, 1925a). The *standard bread* used in all these experiments is described as to ingredients and preparation. This bread contains wheat flour, starch, bran, sugar, cod liver oil, canned tomatoes, canned salmon, yeast and a salt mixture. Bread (S) which contains a little salmon was used in all the experiments here recorded. This bread is a complete diet for an adult dog and will maintain a dog in health for long periods of time, if not indefinitely. This ration keeps the hemoglobin production at a very low level, the average being close to 1 to 3 grams hemoglobin per week period over and above the unknown maintenance factor. There are individual differences and some dogs are constantly high in hemoglobin production on the basal ration as well as on other diets. The weights given for cooked material in all tables represent the weight of the substance *after* cooking and the broth is not added unless so indicated in tables.

All hemoglobin removed from experimental dogs is recorded in the tables. In the first series of experiments we did not include the hemoglobin removed each week to determine the red cell hematocrit and hemoglobin level. This amounts to 1 to 2 grams each week. This explains why the older tables (Whipple and Robscheit-Robbins, 1925a, b; Robscheit-Robbins and Whipple, 1925a, b) often show 0 gram hemoglobin in control periods while the present tables show 1 to 2 grams hemoglobin in similar control periods.

The *hemoglobin index* in these papers is a figure obtained by dividing the hemoglobin per cent by the red cell hematocrit in per cent. We believe this figure gives information of value as to the saturation of the red cell stroma with hemoglobin.

EXPERIMENTAL OBSERVATIONS. In papers II (Robscheit-Robbins and Whipple, 1925a) and III (Whipple and Robscheit-Robbins 1925b) of this series we gave some experimental data on the feeding of beef heart and skeletal muscle. It will be seen that there was considerable variation in the reaction of these two types of striated muscle. In general the beef

TABLE 51

Pig heart feeding

DIET PERIODS 1 WEEK EACH	FOOD CONS.	WT.	PLASMA VOL.	RBC	COLOR INDEX	HB. INDEX	RBC HEMAT.	BLOOD HB. LEVEL	HB. REMOVED BLED
Food, grams per day	*per cent*	*kgm.*	*cc.*	*mil.*			*per cent*	*per cent*	*grams*
Dog 24–22, Coach, female, adult									
Bread 450, salm. 50	100	13.7	780	4,6	0.46	1.85	22.7	42	**1.0**
Bread 450, salm. 50	73	13.5	708	5,2	0.46	1.94	24.7	48	**1.1**
Pig heart 200, bread 350	73	13.2	677	4,7	0.46	1.83	23.6	43	**13.1**
Pig heart 200, bread 350	77	13.4	685	6,1	0.43	1.97	23.3	46	**16.0**
Bread 450, salm. 50	79	12.8	753	4,9	0.44	1.99	21.5	43	**1.2**
Dog 20–104 Bull, male, adult									
Bread 400, salm. 75	55	9.9	566	5,1	0.43	1.88	23.3	44	**1.2**
Pig heart 200, bread 300	90	10.3	536	5,8	0.45	1.90	25.7	48	**11.6**
Pig heart 200, bread 300	71	10.4	570	5,7	0.44	1.96	24.5	48	**14.1**
Bread 350, salm. 100	78	10.5	588	4,4	0.45	1.89	21.2	40	**1.1**
Dog 24–25 Coach, male, adult									
Bread 500	100	14.1	776	4,5	0.40	1.73	20.3	36	**13.0**
Bread 500	100	14.2	820	4,9	0.40	1.79	22.0	39	**1.3**
Pig heart 250, bread 350	100	15.2	830	5,6	0.43	1.84	26.3	48	**1.3**
Pig heart 250, bread 300	100	15.5	775	4,9	0.46	1.80	25.0	45	**18.9**
Bread 500	100	15.8	798	5,4	0.44	1.89	25.3	48	**1.4**
Bread 400	100	15.4	740	6,8	0.43	1.84	26.0	48	**16.7**

$$\text{Hemoglobin index} = \frac{\text{Hemoglobin per cent}}{\text{Red cell hematocrit per cent}}.$$

heart feeding produced more hemoglobin each week than did liberal feeding of beef skeletal muscle. We observe very much the same reaction to pig muscle feeding whether heart or skeletal muscle was used.

TABLE 52

Pig skeletal muscle feeding

DIET PERIODS 1 WEEK EACH	FOOD CONS.	WT.	PLASMA VOL.	RBC	COLOR INDEX	HB. INDEX	RBC HEMAT.	BLOOD HB. LEVEL	HB. REMOVED BLED
Food, grams per day	*per cent*	*kgm.*	*cc.*	*mil.*			*per cent*	*per cent*	*grams*
Dog 21–23 Bull, female, adult									
Bread 400, salm. 100	84	17.7	932	4,7	0.48	2.14	21.2	45	1.3
Pig muscle 300, bread 200	97	17.8	934	4,7	0.54	2.11	23.2	49	14.2
Pig muscle 300, bread 200	100	18.2	994	5,8	0.52	2.11	22.5	48	20.7
Bread 400, salm. 50	100	18.2	1025	5,2	0.49	2.07	21.8	45	16.0
Bread 400, salm. 50	100	18.2	1070	5,4	0.50	2.11	22.9	48	17.9
Bread 400, salm. 50	100	18.2	1040	5,6	0.47	2.00	23.7	47	18.6
Dog 19–104 Bull, male, adult									
Bread 350, salm. 30	66	11.1	592	5,5	0.40	1.95	22.7	44	1.3
Pig muscle 200, bread 200	100	12.0	625	5,3	0.57	1.82	24.5	45	1.2
Pig muscle 200, bread 200	100	12.7	676	6,0	0.40	1.86	25.7	48	1.7
Bread 350	84	12.7	633	5,9	0.43	1.89	27.1	51	1.3
Dog 24–25 Bull, male, adult									
Bread 500	90	14.5	734	5,9	0.42	1.85	26.9	50	1.3
Pig muscle 300, bread 300	100	15.5	850	5,6	0.47	2.00	25.0	50	12.2
Pig muscle 300, bread 300	98	16.4	850	5,7	0.46	1.97	23.2	46	12.8
Bread 500	83	16.0	914	4,4	0.46	1.97	20.6	41	1.1
Dog 18–114 Bull, female, adult									
Bread 250, salm. 75	66	13.9	722	4,3	0.67	2.29	22.0	50	17.5
Bread 250, salm. 100	95	13.7	780	5,1	0.48	2.24	22.1	49	1.3
Pig muscle 500	100	14.5	876	4,2	0.68	2.38	22.1	53	17.8
Pig muscle 500	100	14.2	765	4,1	0.68	2.31	19.4	45	16.5
Bread 275, salm. 75	50	13.8	800	4,2	0.56	2.13	22.2	47	1.3
Bread 225, salm. 100	84	13.7	702	5,2	0.54	2.34	22.2	52	18.1

The pig hearts were thoroughly cooked in boiling water and the gross epicardial fat and walls of the large vessels cut away before the muscle was ground up in a meat grinder for incorporation in the bread diet mixture. The pig skeletal muscle was obtained usually in cheap shoulder cuts

fresh from the abattoir. After thorough cooking in boiling water the gross fat and connective tissue were cut away before the meat was put through the meat grinder. Just before feeding it was incorporated in the diet mixture of bread and water thoroughly mixed so that the dogs could not pick out any diet portions which appealed especially to their taste.

Table 51 gives three experiments out of a total of four which illustrate the reactions observed. We see that pig heart feeding is somewhat more favorable than pig skeletal muscle as reflected in grams of hemoglobin produced over and above the maintenance level. We observe that these dogs on a pig heart diet regenerate 20 to 30 grams hemoglobin each 2-week-period in excess of the control bread periods. There are no noteworthy fluctuations in plasma volume, color or hemloglobin indices. Only the last experiment (dog 24–25) shows a little "carry over" in hemoglobin regeneration—16.7 grams hemoglobin being removed in the second control week following the heart feeding.

Table 52 gives 4 experiments out of a total of 8 which illustrate the reaction to pig skeletal muscle. We believe that pig skeletal muscle as a rule is somewhat more favorable than beef (refer to tables 25 (Robscheit-Robbins and Whipple, 1925a) and 33 (Whipple and Robscheit-Robbins 1925b)). This comparative test is of interest because of the great difference in muscle hemoglobin which is more abundant in beef than in pig muscle. This may indicate that muscle hemoglobin is of relatively little importance in muscle feeding as influencing blood hemoglobin regeneration. The first dog in table 52 (dog 21–23) shows a very unusual reaction to pig muscle feeding. There is a large initial production (30 grams in 2 weeks) and then a very large "carry over" (45 grams in 3 weeks' control period).

The next experiment (dog 19–104) shows an extreme contrast. There was some hemoglobin regeneration although it does not appear except in the rise of the hemoglobin level from the control level of 44 per cent to 51 per cent—perhaps this would amount to 9 grams hemoglobin. At any rate there was minimal blood hemoglobin regeneration on this diet of 200 grams pig muscle and 200 grams standard bread. At the present time we cannot explain these wide fluctuations due to muscle feeding. It is possible that factors of diet and exercise in the days preceding slaughter may influence the storage within the muscles of these substances which contribute to hemoglobin construction.

The third experiment in table 52 (dog 24–25) shows a moderate reaction following heavy pig muscle feeding,—about 20 grams hemoglobin during the 2 weeks. There was considerable increase in weight and plasma volume. Under such conditions we expect less hemoglobin regeneration as it appears that certain substances are deviated from hemoglobin construction during rapid increase in body weight.

The last experiment (dog 18–114) shows a liberal production of hemo-

TABLE 53

Pig uterus feeding

DIET PERIODS 1 WEEK EACH	FOOD CONS.	WT.	PLASMA VOL.	RBC	COLOR INDEX	HB. INDEX	RBC HEMAT.	BLOOD HB. LEVEL	HB. REMOVED BLED
Food, grams per day	*per cent*	*kgm.*	*cc.*	*mil.*			*per cent*	*per cent*	*grams*
Dog 24–49 Bull, female, young adult									
Fish liver 200, bread 400...	90	14.0	808	5,1	0.46	1.80	26.0	47	**1.2**
Fish liver 150, bread 400...	75	14.4	640	6,2	0.41	1.83	27.7	51	**1.3**
Pig uterus 300, bread 300..	100	15.0	830	7,2	0.42	1.77	28.3	50	**18.9**
Pig uterus 300, bread 250..	100	15.1	805	6,4	0.45	1.84	24.3	45	**14.9**
Bread 500.....	100	15.4	770	6,3	0.44	2.00	23.3	47	**12.5**
Bread 500.....	100	15.6	915	5,4	0.45	1.90	25.8	49	**1.1**
Dog 24–25 Coach, male, young adult									
Bread 450.....	100	12.6	572	6,1	0.43	1.81	29.2	53	**1.3**
Pig uterus 300, bread 300..	100	13.5	737	7,0	0.43	1.83	26.3	48	**19.7**
Pig uterus 300, bread 200..	100	13.3	739	6,4	0.43	1.85	26.5	49	**12.6**
Bread 450.....	100	13.6	742	5,0	0.47	1.90	24.8	47	**1.3**
Bread 450.....	100	13.7	750	5,6	0.47	1.85	27.9	52	**11.3**
Dog 24–26 Bull, male, young adult									
Germanium, bread 450....	50	9.7	595	4,4	0.49	2.00	21.4	43	**1.4**
Pig uterus 250, bread 350..	100	10.8	581	4,7	0.48	1.95	23.4	45	**1.5**
Pig uterus 250, bread 350..	100	10.9	638	5,8	0.49	2.00	24.1	48	**14.0**
Bread 450.....	83	10.8	502	4,5	0.61	2.03	26.9	55	**1.4**
Dog 24–46 Bull, female, adult									
Bread 500.....	80	15.2	870	5,4	0.45	1.86	25.9	48	**1.3**
Pig uterus 250, bread 300..	100	15.3	1000	5,5	0.39	1.90	22.6	43	**11.8**
Pig uterus 250, bread 300..	100	15.2	1010	6,3	0.42	1.83	29.5	54	**11.1**
Bread 500, salm. 50.....	100	15.5	940	5,5	0.54	1.83	25.0	46	**15.5**
Bread 500, salm. 50.....	100	15.0	1046	6,7	0.43	1.94	24.8	48	**11.2**

globin—about 46 grams including the "carry over" as a result of a pure diet of pig skeletal muscle for 2 weeks. There are slight but interesting increases in the color and hemoglobin indices during the heavy meat feeding periods.

TABLE 54

Chicken gizzard feeding

DIET PERIODS 1 WEEK EACH	FOOD CONS.	WT.	PLASMA VOL.	RBC	COLOR INDEX	HB. INDEX	RBC HEMAT.	BLOOD HB. LEVEL	HB. REMOVED BLED
Food, grams per day	*per cent*	*kgm.*	*cc.*	*mil.*			*per cent*	*per cent*	*grams*
Dog 18–114 Bull, female, adult									
Bread 275, salm. 75	50	13.8	800	4,2	0.56	2.13	22.2	47	**1.3**
Bread 225, salm 100	84	13.7	702	5,2	0.54	2.34	22.2	52	**18.1**
Chicken gizzard 250, bread 200	100	14.2	808	3,5	0.79	2.31	23.5	54	**24.4**
Chicken gizzard 250, bread 200	100	14.9	848	3,8	0.76	2.27	23.5	53	**38.6**
Bread 225, salm. 100	97	14.2	808	3,9	0.79	2.34	21.0	49	**29.4**
Bread 225, salm. 100	100	14.5	838	3,3	0.79	2.36	20.7	49	**15.6**
Bread 225, salm. 100	100	13.7	806	3,5	0.80	2.33	21.7	50	**19.0**
Bread 225, salm. 100	100	13.6	800	3,8	0.79	2.06	16.3	33	**28.2**
Bread 225, salm. 100	82	13.3	768	3,0	0.66	2.14	18.3	40	**1.4**
Dog 24–49 Bull, female, adult									
Bread 450	100	17.3	985	5,7	0.38	1.90	23.2	44	**1.2**
Chicken gizzard 300, bread 200	100	17.6	978	7,0	0.48	2.11	30.6	65	**35.9**
Chicken gizzard 300, bread 200	100	17.7	896	6,1	0.53	2.11	25.0	53	**34.3**
Bread 450	85	17.3	908	4,8	0.55	2.10	20.4	43	**13.5**
Bread 450	100	17.0	968	4,9	0.53	2.05	22.2	46	**12.3**
Bread 450	100	16.8	960	5,0	0.47	1.91	24.5	47	**1.4**
Dog 19–104 Bull, male, adult									
Bread 250, salm. 75	100	11.6	636	4,1	0.59	2.22	21.7	48	**1.4**
Chicken gizzard 200, bread 150	100	11.8	618	6,2	0.48	2.21	28.4	63	**33.6**
Chicken gizzard 200, bread 150	100	11.8	652	5,1	0.63	2.19	23.0	50	**20.3**
Bread 250, salm. 75	100	11.8	644	4,9	0.59	2.36	20.3	48	**15.5**
Bread 250, salm. 75	100	11.7	685	4,0	0.61	2.16	22.8	49	**1.4**

$$\text{Hemoglobin index} = \frac{\text{Hemoglobin per cent}}{\text{Red cell hemotocrit per cent}}.$$

Table 53 gives 4 experiments out of a total of 6 which illustrate the reaction to a diet of cooked pregnant uterus of the pig. This material was available because of its collection for experiments in embryology and it was used as one test for the value of smooth muscle feeding in hemoglobin regeneration. This material contained also the uterine mucosa as a complicating factor but the bulk of the cooked material consisted of the hypertrophied smooth muscle of the pregnant uterus. This material is seen to be more favorable than beef or pig skeletal muscle and the total hemoglobin regeneration during 2 week periods averages close to 40 grams more than the control periods. All the experiments in table 53 are pretty uniform except the third (dog 24–26) which shows a low output of hemoglobin resulting from a diet of 250 grams pig uterus and 350 grams standard bread.

We may refer at this time to three published experiments dealing with fresh cooked beef stomach—refer to paper I, table 1 (Whipple and Robscheit-Robbins, 1925a); paper II, table 22 (Robscheit-Robbins and Whipple, 1925a); paper IV, table 41 (Robscheit-Robbins and Whipple, 1925b). These experiments show only a moderate reaction—the production of 10 to 20 grams of hemoglobin per 2-week-period above the control levels. The amounts of tripe fed amounted to 200 to 300 grams per day.

Table 54 gives three experiments out of a total of 6 which illustrate the reaction to a diet of cooked chicken gizzard. Some of these gizzards were used fresh and others kept frozen some time in cold storage but no difference was noted. Gizzards were cut open and the food and tough food sac removed. Much of the tough connective tissue was also cut away leaving the deep red muscle to be cooked thoroughly in boiling water. The broth from this cooking was used with the cooked muscle in preparing the diet but other experiments give no indication that the broth added anything other than an agreeable flavor to the diet mixture.

We note an unusual reaction in the first experiment (dog 18–114). This dog reacts to any diet with greater hemoglobin production than normal. We may observe control periods on standard bread with 5 to 6 grams hemoglobin production per week in contrast to the average of 2 grams. Likewise this dog usually produces more hemoglobin than normal on any of the meat product diets. The reaction to gizzard feeding is extraordinary. There is an output of about 50 grams hemoglobin during the two weeks feeding and of about 70 grams hemoglobin above the high control level as a "carry over" which lasts for 4 weeks. This makes a total output of 120 grams hemoglobin over the control level which we must attribute to the chicken gizzard diet. The last two experiments in table 54 show the standard reaction to gizzard feeding. A diet containing 300 grams gizzard and 200 grams bread during 2 weeks (dog 24–49) causes a high output of 90 grams hemoglobin above the control levels. A diet of

200 grams gizzard and 150 grams bread (dog 19–104) causes an output of 60 grams hemoglobin above the control levels.

We may point out in all these gizzard experiments that there is a tendency for the color and hemoglobin indices to run somewhat above the normal level. This may indicate that in such diet periods there is a somewhat more rapid production of hemoglobin as compared with stroma production. In certain liver feeding experiments we have observed a similar tendency but it seems very difficult to exhaust the stroma producing mechanism of the body.

Discussion. Any person who works with standard anemia dogs and observes the ease with which hemoglobin regeneration can be controlled by diet factors comes to believe that many types of human anemia may be treated to advantage by diet control rather than by other methods. We have observed with great interest the clinical studies of Minot, Fitz, Murphy and Monroe (1926), who have placed many cases of pernicious anemia on diets which are favorable for rapid hemoglobin regeneration. They report surprisingly favorable reactions in the majority of cases and their continued study of such clinical material will be of great interest to physicians and physiologists alike. It is to be hoped that other types of human anemia may be subjected to similar study. One of the difficulties encountered in human and animal experiments is related to appetite and ingestion of requisite amounts of food. Careful preparation and slight modifications of the diet mixtures will do much to overcome these difficulties and it should be borne in mind that a few grams of any potent food are not sufficient but abundant intake of sufficient calories is a necessity.

The term "carry over" from a favorable period of hemoglobin regeneration is used frequently to indicate that during favorable diet periods the body stores somewhere a reserve or "carry over" which is made apparent by a subsequent control unfavorable diet period when the body gives up this stored hemoglobin. Apparently these dogs almost always store away somewhere the parent substances of hemoglobin or the hemoglobin itself when placed on a favorable diet (see tables 52 and 54). This "carry over" can always be demonstrated when the dog is placed on the control bread ration which is unfavorable to hemoglobin production. When the favorable diet lasts a long time, 3 to 6 weeks, we may observe a "carry over" of over 3 weeks. Sometimes a single week of control bread diet will exhaust all this reserve. We do not know in what form this material is stored in the body but believe it very unlikely that it is stored as ripened hemoglobin. It seems more likely that it is stored as some *parent substance of hemoglobin* in the liver, kidney, spleen and muscles. We hope to report work bearing on this point in the near future.

To test *smooth muscle* under these experimental conditions we were forced to use three widely different tissues, beef stomach, pig uterus and chicken

gizzard. No single tissue gives us pure smooth muscle alone but there is extraneous material, the mucous membrane of the stomach and uterus. The chicken gizzard contains very little of a thin mucous membrane. Reviewing the results of the feeding of pancreas, thymus and salivary glands we feel that the hemoglobin increase noted in tables 53 and 54 must be due largely to the smooth muscle. There are many interesting possibilities connected with the observations that chicken gizzard is so potent in the regeneration of hemoglobin. We find that it is almost as favorable a diet factor for hemoglobin production as is beef or pig liver. We may look upon this mass of smooth muscle as constantly active and much more productive of energy than other parts of the intestinal tract. It likewise contains a large amount of hemoglobin stored in the muscle fibres. This may amount to more than 1000 mgm. per 100 grams muscle which we may compare with high values of beef and dog skeletal muscle, 800 to 1000 mgm. The amount and type of this hemoglobin found abundantly in smooth muscle deserves further study. We must assume that muscle hemoglobin is used up during muscular activity and it is possible that in these active smooth muscle fibres are stored parent hemoglobin substances which may be called on to repair used up muscle hemoglobin. We may assume the possibility that such material may contribute to the building of red cell hemoglobin when the material is fed. Sections of the gizzard show interlacing bundles of smooth muscle, some connective tissue and a good deal of elastic tissue. No striations were seen in any muscle fibres examined under the microscope.

SUMMARY

Both striated and smooth muscle are favorable diet factors to produce blood hemoglobin in anemic dogs. Smooth muscle in general is somewhat more favorable than striated muscle.

Arranged in order of potency as diet factors for hemoglobin production we find beef skeletal muscle lowest, next beef stomach, a little higher pig heart, beef heart, pig skeletal muscle and pig uterus but highest of all smooth muscle (chicken gizzard).

Cooked pig heart muscle, 200 to 300 grams daily in diet, will average 20 to 30 grams hemoglobin production per 2-week-period more than the control periods in anemic dogs.

Cooked pig skeletal muscle, 200 to 300 grams daily in diet, will show wide fluctuations in hemoglobin production all the way from 10 to 40 grams per 2-week-period more than the control periods.

Cooked beef stomach, 200 to 300 grams daily in diet, will average 10 to 20 grams of hemoglobin production per 2-week-period more than control periods.

Cooked pig uterus (pregnant), 200 to 300 grams daily in diet, will average

almost 40 grams of hemoglobin production per 2-week-period more than control periods.

Cooked *chicken gizzard* (smooth muscle) 200 to 300 grams daily in diet, will show a remarkably high level of hemoglobin production—*from 60 to 100 grams* per 2-week-period more than control periods.

Dogs kept at an anemia level of one-third normal for 15 months show no abnormalities of red cell production. The red cell and hemoglobin production in the early months are exactly the same as the reaction in the 15th month, provided diet, health and environmental conditions are uniform.

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BLOOD REGENERATION IN SEVERE ANEMIA[1]

VI. Influence of Kidney, Chicken and Fish Livers, and Whole Fish

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The kidney of pig and beef as well as chicken liver can be added to the list of cooked animal products which are most potent diet factors in the regeneration of red cells and hemoglobin during long periods of severe anemia. By contrast fish liver is almost inert and whole fish gives but slight regeneration when added to the diet.

These experiments in all details resemble those tabulated in papers I to V of this series and the same methods and experimental controls were used.

The reader may inquire why the *kidney* should be such a favorable diet factor. This indicates the storage in kidney cells of various substances readily convertible into hemoglobin within the dog's body. The favorable diet response and hemoglobin production following *liver* feeding can be explained on the ground that the liver is concerned with pigment metabolism and the liver would be the logical storehouse for reserve substances which are readily convertible into hemoglobin, constituting a part at least of the "carry over" noted on any favorable diet. This "carry over" into the control periods is well illustrated in table 61 although the favorable diet factor is kidney.

Three facts may be mentioned which may explain in part the *abundant hemoglobin production on a kidney diet.* It is well known that there is as a rule in chronic nephritis a definite secondary anemia. This anemia has been attributed in a vague sort of way to intoxication due to the very products which have injured the kidney parenchyma. This is not a convincing explanation and we suggest the possibility that in some unknown fashion the kidney takes part in conservation of some pigment substances or is one of the warehouses where pigment substances or

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TABLE 61

Pig and beef kidney feeding

DIET PERIODS 1 WEEK EACH	FOOD CONS.	WT.	PLASMA VOL.	RBC	COLOR INDEX	HB. INDEX	RBC HEMAT.	BLOOD HB. LEVEL	HB. REMOVED BLED
Food, grams per day	*per cent*	*kgm.*	*cc.*	*mil.*			*per cent*	*per cent*	*grams*
Dog 24–42 Bull, male, adult									
Bread 600	100	16.0	889	5,7	0.45	1.80	22.9	41	14.3
Bread 600	100	15.9	813	5,4	0.46	1.79	27.6	49	1 3
Beef kidney 300, bread 300	100	16.7	903	5,9	0.45	1.90	31.0	58	18.0
Beef kidney 300, bread 300	100	17.5	934	6,5	0.49	2.05	25.2	52	28.9
Bread 600	100	17.0	880	5,2	0.46	1.95	19.9	39	27.3
Bread 600	100	16.8	918	5,5	0.41	1.86	24.4	45	1.3
Dog 21–23 Bull, female, adult									
Bread 500	100	16.4	896	4,7	0.41	1.79	21.4	38	1.1
Bread 500, salm. 50	91	17.0	881	5,8	0.43	1.89	25.4	48	12.8
Beef kidney 250, bread 250	100	17.2	918	6,9	0.48	1.97	29.8	59	27.4
Beef kidney 250, bread 250	100	17.9	892	6,8	0.50	2.19	24.3	53	36.8
Bread 500	100	18.1	905	4,8	0.51	2.05	21.5	44	28.5
Bread 500	100	16.9	876	5,6	0.48	2.00	23.8	48	19.0
Bread 600	88	17.9	1038	4,1	0.47	1.98	19.4	38	1.8
Dog 18–114 Bull, female, adult									
Fowler's Sol. 0.2 cc.									
Bread 500, salm. 50	90	14.6	808	5,4	0.40	1.85	23.6	44	1.3
Pig kidney 300, bread 100	100	14.4	803	6,3	0.42	1.85	33.7	62	16.7
Pig kidney 300, bread 100	100	14.7	726	5,4	0.72	2.11	33.8	71	33.9
Bread 500, salm. 50	93	14.8	740	6,1	0.67	2.03	27.4	56	32.9
Bread 500, salm. 50	100	15.4	865	5,0	0.57	1.93	25.5	49	14.8
Dog 24–45 Bull, female, adult									
Bread 600	97	17.8	1030	5,8	0.45	1.95	23.2	45	14.0
Bread 600	96	17.8	1000	3,9	0.58	2.22	20.4	45	1.3
Pig kidney 200, bread 350	100	18.5	1058	4,8	0.52	1.94	25.1	49	15.4
Pig kidney 200, bread 300	100	19.2	1064	5,8	0.46	1.92	27.5	53	30.0
Bread 600	100	19.2	1008	5,5	0.52	2.03	22.3	45	16.4
Bread 600	100	19.6	1086	5,0	0.53	2.06	25.4	52	14.6
Bread 600	100	19.3	1085	5,2	0.48	2.04	24.7	50	1.4

potential pigment materials are stored against emergency demands. In advanced kidney disease these normal functions may be disturbed with a resultant anemia.

It will be recalled also that in pernicious anemia we find a deposit of considerable iron-containing pigment in the kidney tubules as well as in the liver cells. One may suspect that the kidney in this disease also is as important in the conservation of iron for the body economy as the spleen which usually receives the credit for such iron conservation.

In this connection we may refer to recent analyses published by Forbes and Swift (1926) which show that the normal beef kidney has a very high iron content, 117 mgm. per 100 grams protein, as contrasted with the spleen, 73 mgm. per 100 grams protein, and liver, 40 mgm. per 100 grams protein. In view of these facts it may be within the realms of possibility that the kidney may be concerned with pigment conservation and storage—a sufficient explanation of the very favorable diet response with abundant hemoglobin production following kidney feeding.

Table 61 gives 4 experiments out of a total of 12 which illustrate the remarkable reaction to diets of cooked *beef or pig kidney*. We observe that an addition of 200 to 300 grams of cooked kidney to the diet will result in the production of much new hemoglobin—an increase of 65 to 100 grams hemoglobin per 2-week-period over the control anemia periods on standard bread. We may refer in addition to 2 other experiments with beef kidney—paper II, table 22 (Robscheit-Robbins and Whipple, 1925a) and paper III, table 32 (Whipple and Robscheit-Robbins, 1925b) of this series. These experiments show the same large increase in hemoglobin production above the control periods. We observe a slight but definite increase in the hemoglobin index in 3 out of 4 experiments in table 61, indicating perhaps a slightly more abundant production of hemoglobin than stroma.

Table 62 gives 3 experiments out of a total of 4 which show that *chicken liver* feeding is as favorable for hemoglobin production as is the well established *beef or pig liver* diet. The net over-production of hemoglobin above control periods is 60 to 110 grams of hemoglobin per 2-week-period. In this table we used the cooked chicken liver prepared in the usual fashion by boiling in water. The diet figures apply to liver material after cooking and draining away the broth. The next table 63 shows that there is no especial merit in the *broth* from this liver cooking added to the liver-bread food mixture.

The first experiment (dog 24–46) in table 62 shows a very large output of hemoglobin with a long "carry over"—in all amounting to about 110 grams hemoglobin above the control period. This extraordinary output of hemoglobin was on a large intake (450 grams) of cooked chicken liver daily. We observe that there is a high hemoglobin index during the liver feeding period and extending through the "carry over" period.

The second experiment (dog 24–22) of table 62 shows a very large output of hemoglobin associated with a smaller diet intake of chicken

TABLE 62

Chicken liver without broth

DIET PERIODS 1 WEEK EACH	FOOD CONS.	WT.	PLASMA VOL.	RBC	COLOR INDEX	HB. INDEX	RBC HEMAT.	BLOOD HB. LEVEL	HB. REMOVED BLED
Food, grams per day	*per cent*	*kgm.*	*cc.*	*mil.*			*per cent*	*per cent*	*grams*
Dog 24–46 Bull, female, adult									
Bread 500, salm. 75	97	19.4	968	4,2	0.55	2.08	22.3	46	**1.3**
Chicken liver 450	100	18.4	906	5,2	0.68	2.26	25.9	58	**40.6**
Chicken liver 450	100	18.3	1014	4,7	0.84	2.27	21.8	50	**40.0**
Bread 500, salm. 75	100	19.1	967	3,6	0.75	2.20	21.2	47	**13.0**
Bread 500, salm. 75	100	19.8	975	3,9	0.64	2.27	18.4	41	**16.1**
Bread 500, salm. 75	98	20.0	997	3,8	0.61	2.25	18.6	42	**11.3**
Bread 500, salm. 75	100	20.1	1015	3,6	0.66	2.41	19.7	47	**1.4**
Dog 24–22 Coach, female, adult									
Bread 325, salm. 75	83	13.2	704	4,2	0.57	2.19	21.9	48	**1.4**
Bread 300, salm. 75	84	13.0	743	4,6	0.53	2.21	20.3	45	**13.3**
Chicken liver 225, bread 225	85	13.1	782	5,0	0.45	2.11	21.4	45	**1.4**
Chicken liver 225, bread 225	87	12.7	703	6,4	0.56	2.06	26.7	54	**33.6**
Bread 300, salm. 75	100	12.8	776	5,2	0.58	1.90	18.6	35	**26.0**
Bread 300, salm. 75	73	11.5	686	4,4	0.55	1.76	15.4	27	**11.2**
Bread 250, salm. 100	95	11.8	891	2,1	0.45	1.66	11.4	19	**1.0**
Dog 24–49 Bull, female, adult									
Bread 450	100	17.0	968	4,9	0.53	2.05	22.2	46	**12.3**
Bread 450	100	16.8	960	5,0	0.47	1.91	24.5	47	**1.4**
Chicken liver 200, bread 250	100	15.9	824	6,5	0.54	2.03	27.8	57	**38.9**
Chicken liver 200, bread 250	100	16.0	914	6,2	0.44	1.83	19.6	36	**41.5**
Bread 450	100	16.5	1048	4,0	0.45	1.95	18.2	36	**1.1**
Bread 450	100	16.6	1051	3,9	0.51	2.10	18.9	40	**1.2**

$$\text{Hemoglobin index} = \frac{\text{Hemoglobin per cent}}{\text{Red cell hematocrit per cent}}.$$

liver—about 60 grams of hemoglobin per 2-week-period above control level. In this dog there was too much blood removed during the second control week after the liver feeding and these bleeding figures may be

5 to 10 grams hemoglobin too high. The dog showed some loss of appetite and weight but the clinical condition was not far from normal. In the following week there was a considerable fall in blood hemoglobin and red

TABLE 63

Chicken liver with broth

DIET PERIODS 1 WEEK EACH	FOOD CONS.	WT.	PLASMA VOL.	RBC	COLOR INDEX	HB. INDEX	RBC HEMAT.	BLOOD HB. LEVEL	HB. REMOVED BLED
Food, grams per day	*per cent*	*kgm.*	*cc.*	*mil.*			*per cent*	*per cent*	*grams*
Dog 24-22 Coach, female, adult									
Bread 400, salm. 50	58	13.1	690	5,7	0.50	2.03	24.1	49	**13.7**
Bread 400, salm. 100	88	13.3	816	4,3	0.49	2.14	19.5	42	**1.1**
Chicken liver 250, bread 250	88	13.9	722	5,4	0.62	2.19	30.4	67	**39.7**
Chicken liver 250, bread 250	75	14.0	747	6,2	0.54	1.97	23.5	46	**39.0**
Bread 350, salm. 75	84	13.4	673	4,0	0.57	1.97	23.5	46	**1.3**
Bread 350, salm. 75	81	12.8	729	5,6	0.55	2.01	25.0	50	**17.8**
Bread 325, salm. 75	83	13.2	704	4,2	0.57	2.19	21.9	48	**1.4**
Dog 24-26 Bull, male, adult									
Bread 350, salm. 75	71	11.2	614	5,5	0.48	1.99	18.4	36	**16.3**
Bread 300, salm. 100	64	10.5	667	4,2	0.48	2.17	18.6	40	**1.1**
Chicken liver 200, bread 250	94	11.3	603	5,9	0.57	2.16	30.3	65	**33.5**
Chicken liver 175, bread 175	75	10.7	594	5,8	0.56	2.14	19.8	42	**32.5**
Bread 250, salm. 75	98	10.6	666	3,6	0.58	2.14	19.8	42	**1.2**
Bread 250, salm. 75	100	10.4	634	4,4	0.59	2.08	22.7	47	**13.0**
Bread 250, salm. 75	100	10.5	624	3,9	0.55	2.05	21.0	43	**1.4**
Dog 24-25 Bull, male, adult									
Bread 375, salm. 75	100	16.1	884	5,0	0.48	1.97	24.2	48	**1.3**
Chicken liver 150, bread 300	100	16.1	882	5,9	0.50	2.10	29.7	62	**35.0**
Chicken liver 150, bread 300	97	15.3	758	6,1	0.51	2.00	25.4	51	**32.5**
Bread 375, salm. 75	100	15.1	836	5,0	0.52	2.16	21.2	46	**12.6**
Bread 375, salm. 75	100	15.3	914	4,5	0.56	1.83	19.2	35	**12.4**
Bread 375, salm. 75	93	15.1	972	3,3	0.55	2.23	16.1	36	**1.2**

cell hematocrit which might have been explained as due to red cell destruction as there was no bleeding. Examination of the plasma volume figures gives the correct explanation of these changes—a true hydremia probably due to severe bleeding. This is one of the many examples noted from

time to time in these experiments of the need of blood plasma volume figures. It also shows that the anemic dog has much greater fluctuations in circulating blood plasma volume than is observed in normal dogs.

Table 63 gives 3 experiments out of a total of 4 which show that the broth from cooked chicken liver adds little or nothing to the liver diet mixture as measured by excess production of blood hemoglobin. If we

TABLE 64

Fish liver feeding

DIET PERIODS 1 WEEK EACH	FOOD CONS.	WT.	PLASMA VOL.	RBC	COLOR INDEX	HB. INDEX	RBC HEMAT.	BLOOD HB. LEVEL	HB. REMOVED BLED
Food, grams per day	*per cent*	*kgm.*	*cc.*	*mil.*			*per cent*	*per cent*	*grams*
Dog 24–25 Coach, male, adult									
Bread 450	100	12.8	712	5,0	0.49	1.81	21.5	38	**11.3**
Bread 450	91	11.5	666	5,2	0.40	1.69	24.5	41	**1.2**
Fish liver 150, bread 350	94	11.8	758	5,0	0.37	1.90	19.3	37	**1.2**
Fish liver 150, bread 350	100	12.1	554	5,8	0.41	1.90	25.3	48	**1.3**
Fish liver 200, bread 350	96	12.5	682	5,7	0.42	1.81	26.5	48	**1.3**
Bread 450	100	12.6	572	6,1	0.43	1.81	29.2	53	**1.2**
Dog 24–22 Coach, female, adult									
Bread 500	100	12.9	686	6,0	0.44	1.87	22.4	42	**13.3**
Bread 500	100	13.1	706	5,6	0.39	1.70	25.9	44	**1.0**
Fish liver 150, bread 350	76	12.7	675	5,4	0.43	1.90	24.4	46	**1.1**
Fish liver 150, bread 350	81	12.5	615	6,0	0.44	1.95	23.5	46	**13.2**
Fish liver 150, bread 350	68	12.6	718	5,4	0.40	1.79	23.8	43	**1.0**
Bone marrow pd. 15, bread 500	100	13.0	578	5,3	0.51	1.97	27.1	53	**1.2**
Bone marrow pd. 15, bread 500	100	13.4	703	6,2	0.54	1.94	30.5	59	**20.3**

$$\text{Hemoglobin index} = \frac{\text{Hemoglobin per cent}}{\text{Red cell hematocrit per cent}}.$$

added this broth alone to the standard bread in control periods it is probable that we could demonstrate a slight increase in hemoglobin output. But we cannot claim that these experiments with cooked liver plus the broth from this cooked material show any more remarkable hemoglobin production than do the experiments with cooked liver without broth. These three experiments show an excess hemoglobin production of 70 to 90 grams per 2-week-period over the control standard bread periods.

Table 64 gives 2 experiments which show that *fish liver* is almost inert as measured by diet experiments and hemoglobin production. The fish liver fed in these experiments came from a large fish dealer who handles a variety of large salt water fish. The livers obtained from a variety of sea fish (codfish, haddock, hake and pollack) were removed promptly and

TABLE 65

Whole fish (perch)

DIET PERIODS 1 WEEK EACH	FOOD CONS.	WT.	PLASMA VOL.	RBC	COLOR INDEX	HB. INDEX	RBC HEMAT.	BLOOD HB. LEVEL	HB. REMOVED BLED
Food, grams per day	*per cent*	*kgm.*	*cc.*	*mil.*			*per cent*	*per cent*	*grams*
Dog 24–22 Coach, female, adult									
Bread 350, salm. 50	100	13.0	743	5,4	0.37	1.75	22.5	39	2 2
Bread 350, salm. 50	97	13.0	712	5,8	0.41	1.85	25.5	47	1.3
Fish 200, bread 300	96	13.0	722	5,6	0.48	1.85	20.4	38	11 2
Fish 200, bread 300	78	12.3	690	5,2	0.42	1.94	23.0	44	1.2
Bread 350, salm. 50	82	12.1	710	5,5	0.43	1.94	24.5	47	1.3
Dog 24–25 Bull, male, adult									
Bread 400	100	14.2	768	6,1	0.37	1.66	27.5	46	1.2
Bread 400	100	14.2	708	6,1	0.36	1.74	25.0	43	13.8
Fish 300, bread 300	98	15.2	777	5,5	0.43	1.89	25.2	48	1.3
Fish 250, bread 250	100	15.3	756	6,2	0.44	1.78	23.7	42	11.8
Bread 400	100	15.3	836	5,4	0.42	1.83	24.8	45	1.2
Dog 24–49 Bull, female, adult									
Bread 500, salm. 50	95	15.4	822	6,4	0.37	1.75	26.8	47	1.3
Fish 250, bread 300	100	15.6	876	6,3	0.42	1.82	23.5	43	14 4
Fish 250, bread 250	85	15.3	887	5,4	0.44	1.97	24.1	48	1.4
Bread 400, salm. 75	74	15.0	893	6,3	0.40	1.90	24.3	46	12 9
Bread 400, salm. 75	100	14.9	850	5,9	0.37	1.88	23.2	44	1.2

shipped to us packed in ice. The mixed liver material was cooked as soon as received and kept in an ice box at 4°C. until used in the feeding experiments. This material was very rich in oil and could not be added to the diet in excess of 150 grams per day as the oily mixture would not be eaten by dogs if larger amounts were added daily.

A lipemia resulted from this diet rich in fat and made accurate plasma

volume determination an impossibility. The plasma volume figures show wide fluctuations as might be expected but these are due to method difficulties.

The first control week in both experiments shows a considerable "carry over" from the preceding diet period which was favorable to hemoglobin production. The last period of the first experiment (dog 24–25) shows a slight increase in hemoglobin above the base line level so that we may say in this experiment there may have been 5 to 10 grams of hemoglobin produced over the standard control period. The second experiment shows a slight amount of hemoglobin produced over the control periods—perhaps 10 grams hemoglobin in all during a 3-week-period.

We may refer also to table 53 in the preceding paper where a third experiment is given which shows no production of hemoglobin above control levels during a 3-week-period of fish liver feeding.

Table 65 gives 3 experiments out of a total of 8 which show the slight influence of fresh water fish in a diet mixture. We may say that 200 to 300 grams cooked whole perch added to the bread diet caused an increase of hemoglobin production amounting to only 5 to 20 grams hemoglobin per 2-week-period above the control periods.

We may refer to much experimental data to show that fish muscle is inert in the diet mixture. Our standard bread with and without canned salmon has been described in detail and it is evident that hemoglobin production on the two diets is practically identical. We use the salmon bread because the dogs prefer this food and will eat larger amounts of it for longer periods. We may refer also to heavy feeding of canned salmon in paper I, table 2 (Whipple and Robscheit-Robbins, 1925a), paper II, tables 21 and 24 (Robscheit-Robbins and Whipple, 1925a) to show that 200 to 300 grams of canned salmon causes no increase in hemoglobin production over the control diet bread periods. We have tested dried codfish, paper II, table 24 (Robscheit-Robbins and Whipple, 1925a) with similar results. It will be observed in some of these tables in this paper that 25 to 75 grams of "salm." or canned Alaska pink salmon are frequently added to the diet mixture to make it more palatable to certain dogs.

All this evidence goes to show that a variety of fish muscle and liver are almost inert in regard to hemoglobin production in anemia dogs. We were much surprised to find that fish liver contained no substances which promote hemoglobin production as this liver material is known to be rich in many important food accessories. Moreover it is reported that very active dogs—huskies and Eskimo dogs—in the Arctic region support life almost wholly on a liberal diet of fish. Largely because of this observation we tested *whole fish* and found that this material was a little more efficient in hemoglobin production than salmon muscle, dried codfish or fish liver. It is our opinion however that the dogs in the Arctic get occasional bits

of viscera from warm-blooded animals—bear, walrus, seal, birds, etc., and these diet accessories have much to do with the maintenance of their blood hemoglobin.

SUMMARY

The *kidney* (pig, beef) is one of the most favorable diet factors to promote rapid red cell and hemoglobin production during periods of severe anemia. The presence of 200 to 300 grams of cooked kidney daily in the diet may cause a production of 60 to 100 grams hemoglobin per 2-week-diet-period over and above the control output during standard bread feeding. It is suggested that the kidney may be concerned with pigment conservation and the storage of potential pigment substances.

Chicken liver is as favorable a diet factor for hemoglobin regeneration as beef or pig liver. A liberal ration of cooked chicken liver containing 150 to 400 grams per day will result in 60 to 110 grams of hemoglobin per 2-week-period over the control diet periods. In many of these experiments there may be a moderate increase in the hemoglobin index indicating possibly that the hemoglobin is being formed somewhat more rapidly than the red cell stroma.

Fish liver by contrast is almost inert as a diet factor in hemoglobin production. We have previously shown that a variety of fish muscle is practically inert.

Whole fish may cause a slight increase in hemoglobin production when fed in liberal amounts. This may amount to 5 to 20 grams hemoglobin per 2-week-period over the control level.

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BLOOD REGENERATION IN SEVERE ANEMIA[1]

VII. Influence of Dairy Products on Hemoglobin Production

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The first experiments in our study of red cell and hemoglobin production as influenced by diet were concerned with *milk* combined with bread, rice, potatoes, etc. These experiments dealt with less severe anemia in dogs during short periods of time yet it was obvious from these experiments published some years ago (C. W. Hooper, F. S. Robscheit and G. H. Whipple, 1920) that milk was a diet factor not favorable for red cell and hemoglobin production during simple anemia.

We have a very large amount of data to show that in severe and sustained anemia, whole *milk* added in large amounts to a standard bread ration, will not modify in any recognizable way the output of red cells and hemoglobin per unit period. It must be admitted that milk as a food in anemia is much inferior to many meat products in the measured capacity to produce hemoglobin under controlled experimental conditions.

Physicians should keep this in mind in the dietary control of simple anemias particularly in children where one may observe anemic conditions treated with a diet rich in milk which is the poorest possible food for rapid blood regeneration. It is easily possible to introduce some of the favorable diet factors into these milk diets which will then supply the body mechanism with suitable raw material for the manufacture of red cells and blood hemoglobin.

Butter and cream in liberal amounts added to a standard diet cause a slight but definite increase in hemoglobin output. These increases above standard control periods are 5 to 10 grams for cream and 10 to 20 grams for butter per 2-week-period. How may we explain this apparent anomaly? There is no protein in the butter yet there is an increase in the

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hemoglobin output when butter is fed. One may not fall back on the fat soluble vitamine which might be a graceful if evasive gesture because the standard bread ration contains abundant cod liver oil rich in this very vitamine. We can scarcely consider seriously the salt content of butter but we may recall that certain salts added to the diet at times may cause an increase in hemoglobin production. This may be explained as due to certain readjustments in internal protein and salt metabolism which free substances capable of fabrication into blood hemoglobin. We suggest that the heavy butter feeding may influence hemoglobin production indirectly in a somewhat similar fashion by some unknown readjustment of internal protein metabolism.

METHOD. The same technique and methods were used in these experiments as in other papers of this series and a careful description has been given. The figures used for milk and cream indicate cubic centimeters rather than grams as in all other diet factors. Whole milk used in these experiments was a high grade pasteurized whole milk bought in the open market. The cream was graded as "coffee" cream and contained approximately 19 per cent butter fat. The cheese used was purchased as "yellow American cheese" and contained approximately 38 per cent fat. Butter used was first quality salted market butter made from pasteurized milk. The amounts of cream and butter fed in many instances were quite high, at the very limit of the dogs' tolerance, and it will be noted that the dogs usually gained weight during cream feeding. The salt mixture mentioned in table 73 is the well known salt mixture minus iron citrate used by McCollum and Simmonds (1918).

EXPERIMENTAL OBSERVATIONS. In many of the tables we show experiments to test more than one dairy product on the same dog. In this way we are able to get strictly comparable estimates of the value of various dairy products as influencing hemoglobin production. Table 71 shows a satisfactory experiment with whole milk (450 cc.) and standard bread (350 grams). The fore and after control periods are satisfactory and there is no evidence that whole milk feeding has influenced hemoglobin regeneration in the least. This experiment is typical of very many which we could present but it is not necessary. Refer also to table 73.

Whole milk even when fortified with 100 cc. of cream will cause very little increase in hemoglobin production above the control levels. In table 71 are 2 experiments of this kind and one shows a rather high output on a diet of cream and milk but we must recall that this dog reacts more actively than normal to all diet factors including the control bread diet.

Table 71 also shows a comparison between a diet of milk with cream and a diet of milk with cooked beef muscle. On a general average we will see that the beef muscle will come out ahead but we recall again the fact that beef is the diet factor which is most variable in its reaction in anemic dogs.

TABLE 71

Whole milk, cream and beef skeletal muscle

DIET PERIODS 1 WEEK EACH	FOOD CONS.	WT.	PLASMA VOL.	RBC	COLOR INDEX	HB. INDEX	RBC HEMAT.	BLOOD HB. LEVEL	HB. REMOVED BLED
Food, grams per day	*per cent*	*kgm.*	*cc.*	*mil.*			*per cent*	*per cent*	*grams*
Dog 21–23 Bull, female, adult									
Bread 500	85	17.0	786	4,3	0.46	1.86	21.5	40	**16.8**
Bread 500	100	17.1	913	5,7	0.43	1.84	26.1	48	**1.3**
Milk 450, cream 100, bread 500	100	18.4	1040	5,2	0.37	1.72	21.6	37	**15.8**
Milk 450, cream 100, bread 500	100	19.0	1070	4,8	0.45	1.80	23.8	43	**14.0**
Bread 500	92	19.0	1050	5,3	0.51	1.75	21.6	38	**14.6**
Milk 225, beef 250, bread 150	100	19.2	1200	4,7	0.39	1.69	21.8	37	**10.7**
Milk 225, beef 250, bread 150	100	19.2	1095	5,9	0.36	1.72	24.8	43	**1.4**
Dog 19–104 Bull, male, adult									
Bread 400, salm. 30	100	10.0	552	3,8	0.42	1.74	18.3	32	**1.2**
Milk 450, bread 350	100	10.7	630	5,2	0.39	1.83	22.1	40	**1.4**
Milk 450, bread 350	97	11.4	634	5,7	0.38	1.90	22.9	44	**1.2**
Bread 350, salm. 30	93	11.0	612	5,5	0.43	1.90	24.5	47	**1.5**
Bread 350, salm. 30	85	11.6	650	6,6	0.37	1.90	25.7	49	**1.7**
Milk 450, cream 100, bread 350	74	11.5	630	6,4	0.40	2.00	23.0	46	**11.5**
Milk 450, cream 100, bread 350	52	11.6	680	5,3	0.40	1.76	24.0	42	**1.3**
Milk 200, beef 150, bread 150	100	11.4	685	6,0	0.32	1.81	21.5	39	**1.4**
Milk 200, beef 150, bread 150	100	11.8	653	6,7	0.36	1.83	26.3	48	**1.3**
Milk 200, beef 150, bread 150	100	12.0	658	6,2	0.41	1.76	25.4	45	**11.8**
Bread 350, salm. 30	90	11.8	653	6,6	0.42	2.00	22.1	44	**12.0**
Bread 350, salm. 30	66	11.1	592	5,5	0.40	1.95	22.7	44	**1.3**

$$\text{Hemoglobin index} = \frac{\text{Hemoglobin per cent}}{\text{Red cell hematocrit per cent}}.$$

TABLE 72

Whole milk, cream and beef skeletal muscle

DIET PERIODS 1 WEEK EACH	FOOD CONS.	WT.	PLASMA VOL.	RBC	COLOR INDEX	HB. INDEX	RBC HEMAT.	BLOOD HB. LEVEL	HB. REMOVED BLED
Food, grams per day	*per cent*	*kgm.*	*cc.*	*mil.*			*per cent*	*per cent*	*grams*
Dog 20–104 Bull, male, adult									
Bread 400, salm. 50	65	10.8	686	5,5	0.45	1.92	25.6	49	1.2
Bread 400, salm. 50	65	10.8	554	6,7	0.36	2.00	23.9	48	1.3
Milk 450, cream 100, bread 350, salm. 50	59	10.5	600	6,7	0.38	1.82	22.6	41	13.8
Milk 400, cream 100, bread 300, salm. 50	60	11.5	630	4,9	0.36	1.80	19.9	36	1.2
Beef 150, milk 200, bread 150	91	10.5	618	5,3	0.37	1.85	21.4	40	1.1
Beef 150, milk 200, bread 150	100	10.7	576	5,6	0.43	1.80	26.6	48	1.2
Beef 150, milk 200, bread 150	100	10.4	559	5,6	0.43	1.87	27.2	51	11.0
Dog 24–46 Bull, female, adult									
Milk 450, cream 100, bread 500	76	14.3	854	5,1	0.47	1.90	25.0	48	17.0
Beef 250, milk 225, bread 150	93	15.2	894	6,5	0.38	1.92	26.0	50	1.3
Beef 250, milk 225, bread 150	100	15.5	910	5,0	0.42	1.81	23.2	42	13.4
Bread 500	80	15.2	870	5,4	0.45	1.86	25.9	48	1.3
Dog 24–45 Bull, female, adult									
Milk 450, cream 100, bread 500	98	14.2	980	4,5	0.52	1.90	24.0	46	1.2
Milk 450, cream 100, bread 500	100	15.0	1030	5,2	0.43	1.97	22.6	45	1.3
Bread 500	100	15.0	1030	4,5	0.53	1.95	24.8	49	12.0
Bread 500	100	14.9	1024	5,6	0.40	1.92	23.3	45	1.3
Dog 20–1 Bull, female, adult									
Milk 450, cream 150, bread 450	100	18.9	1008	5,4	0.40	1.81	24.1	44	1.3
Milk 450, cream 150, bread 450	100	19.5	1024	6,8	0.35	1.75	27.0	47	1.4
Milk 450, cream 150, bread 350	100	19.5	1026	5,4	0.48	1.80	26.3	47	11.9
Bread 550, salm. 30	100	19.5	1000	5,8	0.41	1.84	26.2	48	1.3

Some beef will cause a large output of hemoglobin and other samples will cause a small output as shown in table 71. It is possible that some differences in exercise, diet or general condition before slaughter may be responsible for this great variation in market beef muscle.

TABLE 73

Whole milk, cream and beef skeletal muscle

DIET PERIODS 1 WEEK EACH	FOOD CONS.	WT.	PLASMA VOL.	RBC	COLOR INDEX	HB. INDEX	RBC HEMAT.	BLOOD HB. LEVEL	HB. REMOVED BLED
Food, grams per day	*per cent*	*kgm.*	*cc.*	*mil.*			*per cent*	*per cent*	*grams*
Dog 24–42 Bull, male, adult									
Milk 450, bread 450	100	13.2	816	3,6	0.57	1.95	21.0	41	**1.2**
Milk 450, bread 450	100	13.2	940	3,9	0.47	2.01	18.4	37	**1.3**
Milk 450, bread 450	100	13.4	818	3,8	0.53	2.14	18.4	40	**1.2**
Bread 500	100	13.5	912	4,2	0.55	2.27	20.4	46	**1.3**
Bread 500	100	13.4	833	4,8	0.46	2.12	20.8	44	**1.2**
Salt mixt. 15, bread 500	100	13.5	710	4,8	0.52	2.42	20.6	50	**1.3**
Salt mixt. 15, bread 500	100	13.5	886	...	....	1.67	23.2	39	**1.1**
Bread 500	100	13.5	671	4,3	0.45	1.75	22.3	39	**12.0**
Bread 500	100	13.6	680	5,0	0.43	1.83	23.5	43	**1.2**
Milk 450, cream 100, bread 500	100	14.6	774	5,1	0.44	1.70	26.2	45	**1.3**
Milk 450, cream 100, bread 500	100	15.8	854	5,6	0.44	1.78	27.6	49	**1.1**
Milk 225, beef 250, bread 150	100	16.4	994	5,8	0.41	1.84	26.1	48	**1.2**
Milk 225, beef 250, bread 150	100	16.5	891	7,1	0.43	1.76	27.4	48	**21.5**
Bread 500	100	16.2	862	5,8	0.44	1.95	23.9	47	**13.6**
Bread 550	100	16.1	920	5,0	0.42	1.84	23.0	42	**1.3**

The second experiment in table 71 (dog 21–23) shows a high figure of hemoglobin removal in the very first control period which is due to a favorable diet "carry over" from the preceding 2 weeks.

Table 72 gives 4 experiments out of a total of 8 which show a pretty uniform reaction to milk (450 cc.) and cream (100 to 150 cc.). On the average these cream and milk periods run about 10 grams hemoglobin produced per 2 weeks over and above the control periods on standard bread. In all instances there is some gain in body weight.

These cream, milk and butter experiments all show a *color index and hemoglobin index* which tend to fall below the control periods. This is not an absolutely constant finding and the change is not striking but this general tendency we believe is significant and may indicate that the mater-

TABLE 74

Fresh market, salted butter

DIET PERIODS 1 WEEK EACH	FOOD CONS.	WT.	PLASMA VOL.	RBC	COLOR INDEX	HB. INDEX	RBC HEMAT.	BLOOD HB. LEVEL	HB. REMOVED BLED
Food, grams per day	*per cent*	*kilm.*	*cc.*	*mil.*			*per cent*	*per cent*	*grams*
Dog 24–26 Bull, male, adult									
Bread 450	100	10.3	606	4,6	0.50	1.88	24.5	46	**1.4**
Bread 450	100	10.5	565	6,3	0.44	2.03	22.0	45	**15.0**
Butter 100, bread 350	95	10.6	517	4,6	0.46	1.95	21.8	42	**1.9**
Butter 100, bread 350	97	10.8	524	5,1	0.50	1.96	20.5	40	**12.8**
Bread 450, salm. 50	85	10.9	628	5,1	0.39	1.94	20.5	40	**1.7**
Dog 24–25 Coach, male, adult									
Bread 450	96	14.1	646	6,8	0.45	1.91	26.6	51	**12.9**
Bread 450	100	13.7	712	5,9	0.38	1.77	25.5	45	**1.3**
Butter 50, bread 400	100	13.9	739	5,6	0.43	1.82	26.2	48	**1.3**
Butter 50, bread 400	100	14.1	656	6,9	0.41	1.90	25.9	49	**16.3**
Bread 500	100	14.1	776	4,5	0.40	1.73	20.3	36	**13.0**
Bread 500	100	14.2	820	4,9	0.40	1.79	22.0	39	**1.2**
Dog 20–1 Bull, female, adult									
Bread 500	94	19.8	870	5,9	0.53	1.85	24.1	45	**20.6**
Bread 500, salm. 50	100	20.5	1038	4,9	0.47	1.90	24.0	46	**1.3**
Butter 50, bread 400	100	20.4	928	4,9	0.51	1.87	23.7	44	**12.7**
Butter 50, bread 400	94	20.4	958	5,7	0.44	1.82	26.5	48	**13.2**
Bread 500, salm. 50	93	20.4	1020	4,5	0.47	1.90	22.1	42	**1.1**
Bread 500, salm. 50	99	20.6	990	4,8	0.50	1.90	25.3	48	**1.3**

$$\text{Hemoglobin index} = \frac{\text{Hemoglobin per cent}}{\text{Red cell hematocrit per cent}}$$

ials for construction of stroma are more abundant in these diets than are the construction materials for hemoglobin production.

We have two more experiments (table 72) with beef muscle and milk to compare with cream and milk. The difference is not remarkable.

TABLE 75

American cheese and fresh market salted butter

DIET PERIODS 1 WEEK EACH	FOOD CONS.	WT.	PLASMA VOL.	RBC	COLOR INDEX	HB. INDEX	RBC HEMAT.	BLOOD HB. LEVEL	HB. REMOVED BLED
Food, grams per day	*per cent*	*kgm.*	*cc.*	*mil.*			*per cent*	*per cent*	*grams*
Dog 24–45 Bull, female, adult									
Butter 50, bread 500	100	15.6	946	5,8	0.49	1.96	22.9	45	**18.6**
Butter 50, bread 500	100	16.1	930	4,6	0.46	1.88	22.6	42	**1.2**
Bread 600	100	16.0	1032	5,4	0.45	1.96	24.5	48	**13.0**
Bread 600	100	16.2	968	4,8	0.45	1.90	23.0	44	**1.2**
Cheese 100, bread 400	100	17.0	1027	5,1	0.47	2.04	23.6	48	**1.3**
Cheese 100, bread 400	100	17.6	1133	4,8	0.39	1.90	19.9	38	**12.2**
Bread 600	100	18.1	1046	4,9	0.42	1.85	22.3	41	**1.1**
Bread 600	100	17.9	1066	5,9	0.46	1.99	28.4	56	**14.9**
Dog 24–46 Bull, female, adult									
Cheese 100, bread 500	100	15.5	940	4,7	0.45	1.95	21.9	43	**1.3**
Cheese 100, bread 500	100	16.6	1070	5,2	0.41	1.85	23.4	43	**1.3**
Bread 600	100	16.6	1035	5,8	0.40	2.07	22.2	46	**13.1**
Bread 600	100	16.6	992	5,7	0.42	1.89	25.2	48	**1.3**
Butter 75, bread 450	100	16.4	976	5,8	0.42	1.91	25.7	49	**1.4**
Butter 75, bread 400	100	16.8	960	5,7	0.38	1.82	23.7	43	**12.1**
Dog 24–49 Bull, female, adult									
Bread 500	100	15.3	900	4,7	0.50	1.74	27.1	47	**1.3**
Cheese 100, bread 450	100	16.1	920	5,1	0.43	1.79	24.5	44	**1.3**
Cheese 100, bread 450	100	16.7	854	5,7	0.46	1.94	25.0	48	**12.9**
Bread 500	100	16.5	892	4,9	0.40	1.78	21.9	39	**12.8**
Bread 500	100	16.6	907	5,3	0.39	1.81	23.0	42	**1.3**
Butter 100, bread 350	100	16.6	838	5,6	0.42	1.82	25.9	47	**1.3**
Butter 75, bread 300	88	16.2	842	5,0	0.41	1.72	23.5	41	**15.1**
Bread 500	100	16.6	808	5,2	0.42	1.83	24.3	44	**1.3**

Table 73 shows an uninterrupted series of weekly diet periods on a single dog. The milk feeding periods and milk cream periods show no increase in hemoglobin production over the control periods. The addition of beef muscle gives a favorable reaction and we observe the production of

about 30 grams of hemoglobin over and above the control periods, including the "carry over" into the first control period. The salt mixture used is the familiar mixture of McCollum and Simmonds (1918) but without the usual iron citrate. We notice certain fluctuations of plasma volume during these salt periods and the following control periods which may be due to the salt intake.

Table 74 shows 3 experiments out of a total of 10 which illustrate the uniform reaction which follows the addition of butter to the control diet mixture. There are additional butter experiments in table 75. We may say that butter added to the standard bread ration in amounts of 50 to 100 grams daily will cause an increase in hemoglobin production of 10 to 20 grams per 2-week-period over and above the control periods. All the experiments in table 74 show excess hemoglobin production in the first control periods due to favorable diet factors which preceded these control periods—that is, there is a "carry over" of hemoglobin material from the favorable diets into the following control periods. This has been illustrated numberless times in other tables and comes out most conspicuously in the most favorable diets—for example, liver, kidney, etc., see paper VI. It is not very easy to explain why butter can cause an increase in hemoglobin output but this point is discussed early in this paper.

We may refer to an experiment in table 1, paper I of this series (Whipple and Robscheit-Robbins, 1925a) which is of interest because there was exactly 1 year's interval between the identical experiments on the same dog (dog 20–1, table 74). The reactions in the two experiments were practically identical as to the production of hemoglobin per 2-week-period.

Table 75 gives 3 experiments out of a total of 5 which show that "American cheese" added to the diet causes a slight over-production of hemoglobin as compared with control diet periods. On the average the feeding of cheese is about as efficient as the feeding of butter. This table gives adequate control by experiments on the same dogs with butter feeding. It may be argued with propriety that the favorable reaction to a cheese diet may be due largely if not wholly to the butter fat contained in the cheese.

In our earlier experiments with less severe anemia and shorter periods of observation we found a more favorable reaction to butter feeding but the severe anemia is a much more accurate test and gives the true value of these various diet factors.

SUMMARY

Whole milk stands at the foot of the class of diet factors which bring about rapid hemoglobin regeneration in severe anemia. Excellent as milk is for most dietary requirements it is conspicuously inadequate in hemoglobin producing ingredients. Physicians should keep this in mind in diet control of anemia, especially in young children or infants where milk is apt to be the main diet constituent.

Butter fat and *cream* (50 to 100 grams per day) in a standard anemia diet will cause a production of 10 to 20 grams hemoglobin per 2-week-period over and above the control output during standard bread feeding. This is only a slightly favorable reaction and does not compare with favorable diet factors—see paper VI.

Cheese has about the same value for hemoglobin production as butter in standard anemia experiments.

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THE DIURETIC-ANTIDIURETIC ACTION OF PITUITARY EXTRACT

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This paper represents a continuation of previous work from this laboratory on the effect of pituitary extract on the secretion and composition of the urine (1). In this previous work the extremely marked action of posterior lobe extract on the secretion of chlorides was demonstrated. It was also shown that when the volume of secretion per minute was proceeding normally, that is, when there was no increased flow due to the preliminary administration of water, pituitary extract caused a very definite diuresis, but that when the volume was increased by the preliminary administration of water, pituitary extract caused an anti-diuretic effect. The chloride increase occurred under both of these conditions.

The interpretation we are inclined to give to these results is as follows. The diuretic action of pituitary extract (this diuresis can be demonstrated without any doubt whatever under the conditions we have described) is a "salt" effect. We believe it to be due to the discharge of salts from the tissues into the blood, these being promptly secreted; thus the composition of the blood may be kept constant. Obviously these salts must be secreted in solution and the diuresis observed represents nothing more than solvent for them. We are aware that other explanations have been given to explain this diuretic action. For example, the increase in kidney volume which follows the administration of pituitary extract could be taken to represent an increased blood flow (a fact demonstrated directly by Cushny and Lambie (2)) which has been suggested as explaining the diuresis. This explanation implies that the action of pituitary extract is upon the kidney itself but we prefer the view that this vascular effect is merely the result of the kidney's effort to eliminate the excess of salts brought to it as the result of the action of pituitary extract in the tissues. This point will be developed later. We do not wish to state that pituitary extract has no action whatever on the kidney but rather to leave this side of the question open. Our reason for this is chiefly the result of the experiments of Starling and Verney (3) who, using a heart, lung, kidney preparation, observed an increased chloride secretion and concentration

after pituitary extract. Even in these experiments, however, the small effect observed may have been due to the discharge of salts from the tissues present into the circulating fluid.

A diuretic action of pituitary extract cannot be shown when the rate of urine secretion is high initially. This has been amply demonstrated by numerous workers. The reason that an increased diuresis does not develop under this condition lies in the fact that the excretion of water is already so voluminous that the salts, which are excreted in greatly increased amount in this condition also, require no additional solvent for their elimination. In truth, the amount of water secreted can be, and actually is, diminished by pituitary extract without impairing the capacity to rid the blood of its excess of salts; consequently an additional diuresis does not develop.

In our earlier paper we recognized the diuretic action as being possibly due to the mechanism sketched above, but we stated there that we preferred to regard it as due to a definite stimulating action on the secretion of water by the kidney and the antidiuretic action to an inhibitory action on the secretion of water by the kidney. We now incline, however, to the explanation suggested above for reasons to be given soon. The basis for the earlier view lay in the failure to observe significant blood changes under the influence of pituitary extract so that it was felt necessary to assign the point of pituitary attack to the kidney. The experiments of Starling and Verney just mentioned also influenced our viewpoint. An additional reason existed in the fact that when the secretion of chloride was previously increased by the administration of sodium chloride, pituitary extract did not cause an additional increase in chloride concentration; it caused rather a decrease though the minute rate of chloride excretion was increased. We have no real explanation of this result.

The foundation for our present views lies in the results contained in our earlier paper and those herein contained. An attempt has been made to locate the point of pituitary attack by carrying out experiments similar to those already described but examining the urine for its sodium and potassium content in addition. Dogs with bladder fistulas were employed so that the results are uncomplicated by the action of an anesthetic. Chlorides were in some cases determined volumetrically by the usual method and in other cases gravimetrically after digesting the urine with strong nitric acid containing silver nitrate and then filtering and weighing in Pregl tubes. Sodium and potassium were determined by the perchlorate method of Smith and Ross (4) which we found entirely satisfactory on known solutions.

Our hypothesis was as follows: The blood of the dog, according to the analysis of Abderhalden, contains 14 mgm. of potash in the serum and 11 mgm. in the corpuscles of 100 mils of blood. Of soda it contains 239 and 127 mgm. respectively. If now pituitary extract acts primarily upon the kidney one would expect the urine secreted following its administration to

have about the same ratio between the sodium and potassium contents as the control urine. Since the urine is presumably derived from the plasma this view would appear reasonable. On the other hand, if pituitary extract acts more remotely, say in the tissues generally, it might be expected that the sodium: potassium ratio might vary. Unlike blood, tissue cells are richer in potassium than sodium; consequently if the action is in the tissues and if the sodium and potassium of the urine following pituitary extract are derived from them, the potassium excretion should be more affected than the sodium excretion. The following protocols speak for themselves. Considering that blood serum contains seventeen times as much soda as potash and that the control urine always contains less potassium than sodium (in some cases very much less) the fact that after pituitary extract the potassium excretion exceeds that of sodium seems to indicate clearly that the point of attack of pituitary extract is in the tissues. This state of affairs is soon reversed and sodium excretion again becomes dominant, but nevertheless the initial change which occurs is remarkable. The alternative view that pituitary extract affects the secretion of potassium by the kidney enormously more than it affects the secretion of sodium does not seem a likely explanation.

In two experiments (only one presented here) we have studied the Ca and Mg excretion and found these elements also to be augmented by pituitary extract. Ordinarily the quantity of calcium in dog's urine is small. On a diet similar to that employed in these experiments the excretion amounts to about 20 mgm. per day. In the control urine collected over a period of about thirty minutes we have consequently found Ca and Mg barely detectable but after the extract the rate is increased to such an extent as to be quite measurable. The method of McCrudden (5) was employed and while only an approximation can be claimed for the control rate the accuracy in the experimental periods is sufficient to indicate a great increase in the calcium and magnesium excretion.

The effect of pituitary extract upon phosphorus excretion also supports the belief in a tissue effect. Consultation of our earlier paper will show that in one case the excretion of phosphorus was increased at least 6-fold and in other experiments decided increases were observed. In experiment 7 of this series the increase was 48-fold. Muscle tissue is very high in phosphorus and may be regarded as a much more likely source of this element than blood, which contains only a few milligrams of inorganic phosphorus per 100 cc. It will be noted in the protocols that the quantities of chlorine excreted are usually less than the amounts corresponding to the amounts of potassium and sodium. The deficiencies are most pronounced in the post-pituitary periods and possibly represent increased excretion of phosphoric acid. The augmented secretion of urea which we were previously inclined to regard as indicating a direct stimulation of kidney function

by pituitary extract, we believe can be regarded as a secondary phenomenon. The activity of the kidney is in all likelihood increased through the necessity thrown upon it of excreting the increased quantities of potassium and sodium brought to it and it appears reasonable that this increased activity results in an augmented urea excretion.

The idea that the urinary phenomena observed to follow pituitary extract administration are the result of an extra-renal action has been suggested by a number of investigators. Miura (6) describes a diminution in the hemoglobin content of the blood and an increase in the chloride content of the blood thus pointing to a hydremic action. Underhill and Pack (7) have also observed a blood dilution but ascribe the slight diuresis (slight, compared with what they say would be expected in view of the high degree of hydremia) to "diminished sensitiveness of the kidney to the hydremic stimulus." We do not think, however, that this result necessarily implies involvement of the kidney. The hydremia may be the result merely of the presence of an increased amount of electrolytes in the blood but it does not follow that this hydremia is due to the inability of the kidneys to secrete water. Indeed our experiments show that it is decidedly able to secrete water for the diuresis in some cases is quite pronounced; it is much greater than Underhill and Pack estimate. They state, for example, that the normal secretion of urine is 2 to 8 cc. per hour, which is augmented by pituitary to only 8 to 20 cc. hourly. In a 10-kilogram dog our experiment 7 shows the rate at one period to be 115 cc. hourly or 2760 cc. per day, a rate which, considering the animal's size, is certainly pronounced. Moreover, in this experiment the control rate was only 0.079 cc. per minute. If the kidney was the only organ involved after an hour of anuria, only 5 cc. of water would have been withheld in the body. This would dilute the blood say 1 per cent and could not possibly account for the hydremia which Underhill and Pack describe. Obviously, as Underhill and Pack emphasize, there is some extra-renal factor.

Supporting the extra-renal idea Miura also found that when the extract was injected into the left renal artery diuresis ensued from the right kidney in most cases as soon as from the left—the left showed the initial short anuria sooner than the right. In our experiments we have always observed a short period of anuria[1] to be the immediate result of pituitary extract ad-

[1] In the second edition of his monograph on "The Secretion of the Urine" Cushny states that the initial anuria following the injection of pituitary extract had been confused by the author with the antidiuretic action This is not the case as is sufficiently evident from the following sentence which occurs in the paper cited by Cushny: "whether one observes diuresis or antidiuresis depends upon the rate of secretion at the time—when it is high the antidiuretic action predominates, but when it is low diuresis ensues." It is also made abundantly clear that the initial anuria or oliguria occurs whether or not the main effect is diuresis or antidiuresis.

ministration, but we have not been greatly concerned with this phase of pituitary extract action. One possible explanation, namely, ureteral spasm, as suggested by Molitor and Pick (8), appears plausible in view of the experiments of Mackersie (9), who did not observe anuria after inserting a catheter into the hilus of the kidney. However, it is difficult to reconcile this result with some experiments of Miura who, as just stated, found the anuria to occur sooner in the left kidney when the injection was made into the left renal artery. This would indicate a kidney effect rather than an action on the ureters. The decrease in the number of active glomeruli after pituitary extract described by Richards and Schmidt (10) also points to a direct kidney effect. Whatever the explanation may be, the phenomenon would not seem to have anything to do with either the diuretic or the extended anti-diuretic actions.

In passing it seems desirable to make some comment upon the experiments of Knowlton and Silverman (11) who could detect no increased oxygen consumption in pituitary diuresis. It is impossible to reconcile this result with our own data. Following pituitary extract the astonishing increase in chloride concentration indicates the performance of work done by the kidney. The chlorine concentrations sometimes reached are from two to three times that of the blood plasma, concentrations which, of course, cannot be produced without the expenditure of energy.

The results of the experiments here reported tempt one to try to relate them to the rôle of the pituitary in diabetes insipidus. The outstanding derangement in this disease is the inability of the organism to retain water so that marked thirst and diuresis result. The fundamental cause of this inability to retain water is, of course, unknown, but we should like to suggest that it may be associated with a disturbance in the electrolyte content or in the balance of ions in the tissues. Our data show that there is a very pronounced change in the excretion of electrolytes and it may be that the loss of electrolytes by the tissues or a change in the ratios of say potassium and sodium in the tissues which may result from the administration of pituitary extract alters the capacity of the tissues to retain water. That the water balance is affected by pituitary extract has been shown by a number of investigators. Weir, Larson and Rowntree (12) demonstrated that in dogs, if large amounts of water and pituitary extract are given concomitantly, water intoxication develops, and Priestley (13) found water diuresis in men to be inhibited. Biasotti (14) showed that frogs injected with pituitary extract absorb water to the extent of 15 per cent of their body weight, the muscles and subcutaneous tissues becoming edematous.

Data which bear directly upon the idea suggested here are lacking in the literature but it is known that the permeability of cells is affected by the nature of the ions contained in the bathing medium. We do not know from the results of our experiments whether or not the ion balance is altered

enough to affect cellular permeability or the capacity to retain water nor do we know that the capacity to retain water is actually affected by the loss of salts indicated in these experiments, but it is not impossible that such may be the case. In this connection the experiments of Hauberrisser and Schonfeld (15) are of interest. These investigators have shown that the amount of water imbibed by connective tissue placed in isotonic solutions containing calcium or magnesium chlorides is different from that which results when the solutions contain sodium, potassium or ammonium. Their results show no distinction between sodium and potassium. Gollwitzer-Meier (16) and Kempmann and Menschel (17) have recently shown ion changes to be involved in the production and disappearance of edema.

Assuming that the capacity of the tissues to retain water is a function of the electrolyte content or ion balance of the tissues, we might regard the presence or absence of pituitary extract as entailing the following consequences. Pituitary administration causes the tissues to lose more potassium than sodium. This disturbance increases the capacity of the tissues to retain water, hence the anti-diuretic effect. Absence or lack of pituitary secretion allows the reverse phenomenon to occur. That is, potassium may accumulate to a greater extent than usual. This decreases the capacity of the tissues to retain water, hence the diuresis and thirst of diabetes insipidus. It is not to be understood that sodium and potassium are necessarily the only elements involved. They are singled out because definite information extends to them only aside from a slight knowledge of the phosphorus, calcium and magnesium excretion.

The diuretic effect of pituitary extract offers no point contrary to hypothesis suggested. As a result of the necessity to excrete the potassium and sodium discharged into the blood there may be some competition between the tissues and kidney for water. In this case while the tissues are probably doing their best to retain water the kidney succeeds in obtaining enough to eliminate the salts brought to it. It must be remembered that the urine of pituitary diuresis is a concentrated urine with regard to its salt content. It contains as high as 1 per cent of chlorine, or three times as much as the blood serum of the dog.

In conclusion it seems worth while to suggest that some of the pharmacological actions of pituitary extract may be due to alterations in the quantities or inter-relationships of electrolytes in the tissues.

CONCLUSIONS

The diuretic-antidiuretic action of pituitary extract is chiefly or wholly a secondary result of a primary action in the tissues. This is indicated by the fact that the urine secreted after pituitary administration is extraordinarily rich in potassium, calcium, magnesium and phosphorus, which presumably are derived from tissue cells.

It is suggested that the diuretic action of pituitary extract is purely a salt effect resulting from the necessity of the kidneys to eliminate salts thrown into the blood by the tissues.

The antidiuretic action, which is the chief action of pituitary extracts, is regarded tentatively as explainable on the assumption of an increased capacity of the tissues to hold water as a result of the electrolyte changes.

It is suggested that in diabetes insipidus the failure of the body to retain water is due to a reverse condition regarding electrolyte changes which decreases the ability of the body to retain water.

TIME	VOLUME* PER MINUTE	Cl* PER MINUTE	Cl PER CENT	Na* PER MINUTE	K* PER MINUTE	Calc. Cl* PER MINUTE
Experiment 1						
1:55–2:35	0.38	0.37	0.098	0.258	0.064	0.466
2:37—1 cc. "Pituitrin" intravenously						
2:39–2:49	0.13					
2:49–3:14	0.69	3.58	0.520	1.68	1.65	4.08
3:14–3:29	0.68	2.26	0.333	1.32	1.06	2.99
3:30–3:45	0.64	1.32	0.200	0.835	0.76	1.98
Experiment 2						
2:36–3:13	0.32	0.633	0.202	0.554	0.106	0.951
3:13—1 cc. "Pituitrin" intravenously						
3:13–3:43	0.33	1.67	0.50	0.490	0.958	1.63
3:43–4:06	1.34	7.3	0.55	4.23	1.92	8.26
4:06–4:31	0.67	2.42	0.36	1.56	1.15	3.44
Experiment 3						
1:30–2:00	0.61	0.645	0.106	0.076	0.050	0.162
2:03–1cc. "Pituitrin" intravenously						
2:05–2:45	0.48	1.04	0.215	0.572	0.757	1.57
2:45–3:00	1.53	6.90	0.45	3.84	1.38	7.16
3:00–3:20	0.78	2.46	0.317		0.70	
Experiment 4						
1:45–2:15	0.61	0.342	0.056	0.285	0.139	0.565
2:18—1 cc. "Pituitrin" intravenously						
2:20–2:45	0.23	0.246	0.107	0.259	0.174	0.557
2:45–3:10	0.75	0.550	0.073	0.872	1.07	2.04
3:10–3:30	0.74	0.379	0.051	0.463	0.563	1.225

* Volumes and quantities are in cubic centimeters or milligrams.

TIME	VOLUME PER MINUTE	Cl PER MINUTE	Cl PER CENT	Na PER MINUTE	K PER MINUTE	Calc. Cl PER MINUTE
Experiment 5						
1:55–2:30	0.82	0.524	0.064	0.326	0.051	0.548
2:35–1 cc. "Pituitrin" intravenously						
2:36–3:00	0.15					
3:00–3:20	0.90	1.95	0.217	0.963	1.25	2.61
3:20–3:40	1.43	4.15	0.29	2.08	1.12	4.22
3:40–4:00	1.11	1.78	0.16	0.703	0.623	1.64
Experiment 6						
2:12–3:02	0.147	0.138	0.094	0.182	0.131	0.399
3:04—1 cc. "Pituitrin" intravenously						
3:05–3:13	0.125					
3:13–3:28	0.55	2.49	0.45	1.34	2.72	4.52
3:28–3.45	1.18	4.86	0.41	3.22	2.82	7.51
3:45–4:00	0.59	3:13	0.53	2.83	1.56	5.82
Experiment 7						
TIME	VOLUME PER MINUTE	Cl PER MINUTE	Cl PER CENT	Mg PER MINUTE	Ca PER MINUTE	P PER MINUTE
9:42–10:17	0.079	0.099	0.125	nil	0.011	0.028
10:17—1 cc. "Pituitrin" intravenously						
10:25–10:40	0.62	4.1	0.676	lost	0.190	0.628
10:40–10:52	1.92	12.6	0.657	0.23	0.404	1.32
10:52–11:02	1.70	13.2	0.777	0.18	0.310	1.08

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STUDIES ON BILE-SALT HEMOLYSIS

I. The Fixation of Bile-Salts by Serum as an Absorption Phenomenon

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Most hemolytic agents are inactivated by blood serum. Ransom (1), for example, showed that this was the case in saponin hemolysis and concluded from his researches that the saponin fixation was accomplished by its union with the cholesterol contained in the blood. Bayer (2) and Sellards (3) reported that bile-salt hemolysis was prevented when sufficient serum was added to the system. The protective power of serum in hemolysis by the soaps has been investigated by Noguchi (4), Lieberman (5) and Clark (6). Moss and Barnes (7) have demonstrated the neutralization of cobra venom by serum, Ford (8) has shown that the hemolytic principle of the Amanita Phalloides was inactivated by serum. The problem of the inhibition of the hemolysis produced by saponin and sodium taurocholate has been investigated quantitatively by Ponder (9) in a very extensive and painstaking research. He studied the effects of saponin and sodium taurocholate on the blood both in vitro and in vivo and demonstrated that the intravenous injection and the absorption of these materials from the intestinal tract was followed by a measurable diminution in the inhibitory power of the serum, but not to the extent of causing intravascular hemolysis.

These studies and others have definitely established the fact that blood serum has an inhibitory action on hemolysis. Knowledge is still lacking, however, concerning the specific substances which produce this protection. It was thought that the serum calcium was the substance which protected the corpuscles from hemolysis by the soaps until it was shown that the protective power was but little altered after removal of that metal. While, no doubt, the calcium and other salts of the serum play an important rôle in the reaction, the cholesterol, protein and certain fats are also an essential part of the antihemolytic system. Thus Bayer showed that the protective action of serum against hemolysis by bile-salts was removed after it had been digested with trypsin and erepsin. He believed that the inhibitory

action was due to protein alone. On the other hand, Sellards found that the protective power of serum persisted in good measure even after the removal of protein by heat. The ability of blood serum to fix hemolysin is an important mechanism in protecting the body against certain toxic substances. It is in this way that the dangerous hemolytic action of soaps and bile-salts, which are normally present or which may be increased in the blood following biliary obstruction, is neutralized. Perhaps, on the other hand, in certain of the anemias we are confronted, not with the presence of a foreign hemolytic substance, but by a diminution in the protective power of the serum for substances which are normally present. The nature of the union of the hemolytic material and serum and the factors which cause an alteration in their association are of interest. The present paper is concerned with the quantitative relationships of the bile-salt serum system.

EXPERIMENTAL. A very pure preparation of bile-salts, containing 60 per cent sodium glycocholate and 40 per cent sodium taurocholate, was dried over sulphuric acid. A standard bile-salt solution was then prepared by dissolving a weighed portion of this dry material in a volume of absolute ethyl alcohol, so that the amount of bile-salts required for an experiment could be obtained accurately and quickly by evaporation of a volume of this stock solution. The material to be used in an experiment was prepared by pipetting the volume of the standard solution, which contained the required amount of bile-salts, into a high-walled beaker and evaporating it to dryness over a water bath. The residue was further dried over calcium chloride and then dissolved in sufficient physiological sodium chloride solution to make a 1 per cent solution.

Fresh human blood was used throughout the study. From 25 to 50 cc. of blood were drawn from the arm vein of a normal adult by means of a syringe. Part of this blood, usually 5 cc., was then added to 10 cc. of physiological sodium chloride solution containing 1 cc. of 1 per cent sodium citrate and the remainder was allowed to clot. The serum was withdrawn from the clot, warmed to 55°C. for fifteen minutes and used not later than an hour after its collection. The citrated blood was centrifuged, washed three times with physiological sodium chloride solution, and a suspension made by diluting the corpusles with four times their volume of the salt solution.

Method. The method of making the quantitative measurements of the hemolytic activity of the bile-salts was the same throughout the experiment. Dry test tubes measuring 1 × 10 cm. were placed in a suitable rack and increasing volumes of 1 per cent bile-salt solution were measured into them by means of a microburette graduated to 0.01 cc. Sufficient physiological sodium chloride solution to make the total volume of each tube 5.0 cc. and two drops of the washed corpuscle suspensions were then

added. The tubes were then closed with dry cork stoppers, mixed well and allowed to stand for two hours in a constant temperature oven adjusted to 37°C. The tubes were gently inverted approximately every fifteen minutes to prevent settling of the corpuscles. At the end of two hours the tubes were centrifuged and those containing bile-salts were compared with a control tube which contained only corpuscles and physiological sodium chloride solution. The lowest concentration of bile-salts which would cause the faintest tinge of color in the supernatant fluid was accepted as that which was capable of causing hemolysis.

The inhibitory action of serum. The smallest amount of bile-salts capable of producing hemolysis of corpuscles in the absence of serum was determined on the blood of ten different normal adults. The blood was collected and treated as previously outlined so that both corpuscles and serum were obtained. A series of tubes were then suitably marked and to them

TABLE 1

CASE	HEMOLYSIS IN ABSENCE OF SERUM: BY GRAM OF BILE-SALT	HEMOLYSIS IN PRESENCE OF 0.20 CC. SERUM: BY GRAM OF BILE-SALT
1	0.0015	0.0055
2	0.0012	0.0058
3	0.0018	0 0058
4	0.0010	0.0052
5	0.0015	0.0060
6	0.0015	0.0053
7	0.0012	0.0055
8	0.0010	0.0060
9	0.0015	0.0052
10	0.0015	0.0050

ascending amounts of bile-salts were added. The contents of each tube were then diluted to 5.0 cc., two drops of the emulsion of corpuscles were introduced and the tubes incubated as described. The smallest concentration of bile-salts which produced hemolysis is given in table 1.

The effect of the serum was next studied. The serum, prepared as already noted, was diluted with physiological sodium chloride solution so that 1 cc. of the mixture contained 0.20 cc. of serum. A series of tubes were arranged as before and to each was added 1 cc. of the diluted serum. The bile-salts were then added in progressively larger doses and two drops of the corpuscle emulsion were introduced after each tube had been diluted to 5.0 cc. with the salt solution. The smallest concentration of bile-salts which produced hemolysis in the presence of a 0.20 cc. of serum is given in table 1.

An examination of these data in the table reveals the fact that while the corpuscles are just hemolyzed by approximately 0.0014 gram of bile-salts

in the absence of serum, in the presence of 0.20 cc. of serum there is no hemolysis until 0.0055 gram of bile-salts is added. It is interesting to observe that the inhibitory power of the serum is, within limits, constant for this group of normal persons.

What is the nature of this combination between the serum and bile-salts? From the constancy of the ratio between the two, as determined in the above experiment, it might be concluded that the combination is a chemical one. However, this by itself cannot be taken as an absolute criterion. For example, compare the somewhat similar combinations such as the absorption of acetic acid by charcoal or the mutual precipitation of colloids which also conform to this requirement and in which the

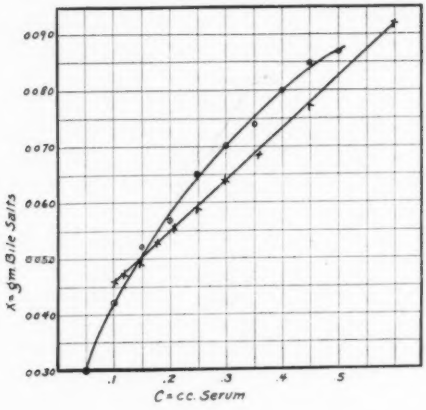

Fig. 1

phenomena cannot be considered to be chemical reactions in the usual meaning of the term.

Is this fixation of bile-salts by serum due to absorption or to a chemical combination? That it is an absorption phenomenon seems probable because the degree of neutralization varies not only with the amounts of the materials present but also with the rate at which the addition is made. If the interaction between bile-salts and serum is a chemical reaction, one would expect that if a given quantity of serum would exactly neutralize a given quantity of bile-salts, the former must be proportionally increased in order to neutralize twice the quantity of the latter. This does not hold true. The amounts of bile-salts necessary to produce hemolysis in the presence of various amounts of serum ranging from 0.05 to 0.50 cc. were determined. The results are shown in the graph and in table 2.

A study of the graph (fig. 1) reveals that while 0.10 cc. of serum neutral-

izes 0.0042 gram of bile-salts, twice the amount of serum neutralizes much less than twice the quantity of the hemolytic agent. Thus instead of 0.20 cc. of serum fixing 0.0084 gram of bile-salts it is actually only 0.0057 gram which is neutralized or when the quantity of serum is increased fourfold not 0.0168 gram but 0.0080 gram is found to be fixed. It is this discrepancy which leads us to the belief that the action between bile-salts and serum may be due to absorption. If this is the case, the quantitative relationships should conform to the formula:

$$\frac{X^n}{C} = K$$

in which X is the total amount of bile-salts absorbed, C is the concentration of the absorbent and n and K are constants.

TABLE 2

C (CC. SERUM)	X (GRAM BILE-SALTS)	$K = \frac{X^{2.175}}{C}$	ΔK
0.05	0.0030	6.51×10^{-5}	-0.27×10^{-5}
0.10	0.0042	6.76×10^{-5}	-0.02×10^{-5}
0.15	0.0052	7.12×10^{-5}	$+0.34 \times 10^{-5}$
0.20	0.0057	6.57×10^{-5}	-0.21×10^{-5}
0.25	0.0065	6.99×10^{-5}	$+0.21 \times 10^{-5}$
0.30	0.0070	6.80×10^{-5}	$+0.02 \times 10^{-5}$
0.35	0.0074	6.63×10^{-5}	-0.15×10^{-5}
0.40	0.0080	6.87×10^{-5}	$+0.09 \times 10^{-5}$
0.45	0.0085	6.98×10^{-5}	$+0.20 \times 10^{-5}$
0.50	0.0087	6.60×10^{-5}	-0.18×10^{-5}

The validity of the equation may be tested by plotting the logarithms of the experimentally determined values of X against the logarithms of the corresponding values of C. If the absorption equation holds, a straight line should result. In the curve above log n is shown as a function of log C.

Since the values of X and C have been determined experimentally, it remains to calculate the values for n and K. The logarithms of both sides of the equation of the absorption isotherm gives the equation of a straight line if log C is plotted as ordinates and log X as abscissa. When these data were plotted on coördinate paper, all of the points lay so nearly on a straight line that the very accurate method of averages was used to determine the precision. The value of n was found to be 2.175, the values of K are given in table 2.

An average value of $K = 6.78 \times 10^{-5}$ was obtained. A comparison of the values for K shows that the absorption formula expresses the experimental results very closely.

The substances concerned in the bile-salt serum interaction are quite certainly colloidal in nature. The fact that the quantitative relationships of the interacting substances conform to the absorption equation strengthens the assumption that we are dealing with a colloidal reaction which is physical rather than chemical. Another similarity between this and the colloid reactions is seen when the manner of the addition of the serum and bile-salts is varied. It will be demonstrated that when a given amount of bile-salts is added in more than one fraction, with a definite period of time beween each addition, more bile-salts remain free than when the entire quantity is added at once.

This phenomenon, known as the Danysz effect, is a well-known characteristic of colloid reactions. In 1902 Danysz (10) found that when diphtheria toxin was treated with anti-toxin, the reduction in toxicity depended on the manner in which it was added. An amount of anti-toxin exactly sufficient to neutralize a given amount of toxin was adequate only when the entire amount was added at one time. If added in fractions, with moderate intervals between the additions, more antitoxin was required. A similar observation was made a year later by Freundlich (11) who found that the quantities of certain suspensoid sols which were precipitated by electrolytes varied with the rate of addition of the electrolytes. The phenomenon was further observed by Gordon (12) in 1904 in the "salting out" of certain emulsoids such as albumin. He found that if all of the salt was added at one time more albumin was precipitated than if it was added gradually. Thus when 5.0 cc. of saturated ammonium sulphate was added in one operation to an equal volume of egg sol there remained 0.17 gram of albumin in the filtrate; on the other hand, when the addition of the ammonium sulphate was spread over a period of twenty-four hours less albumin was precipitated and the filtrate contained 0.214 gram of the albumin.

The effect of the gradual addition of bile-salt to serum. Two sets of test-tubes were arranged in suitable racks and 0.20 cc. of serum diluted to 1 cc. with physiological sodium chloride solution was added to each tube. A 0.5 per cent solution of bile-salts was prepared in the usual way and increasing volumes of this were added to the serum of one set of tubes in the following manner: One-third of the amount of the bile-salt solution required in each tube was added, the tubes were securely stoppered and the resultant mixture allowed to stand in the ice-box for an hour, after which the second third of the bile-salts was added. The tubes were again allowed to stand in the ice-box for an hour when the final third of the bile-salt solution was added. Identical amounts of bile-salts were then added in one portion to the 0.20 cc. of diluted serum of the corresponding tubes of the second set after which both series of tubes were allowed to stand for fifteen minutes. At the end of this time each tube was diluted to a total

volume of 5.0 cc. and the same amount of corpuscles added to each tube. All of the tubes were then placed in an oven at a temperature of 37°C. and occasionally inverted to prevent settling of the corpuscles. At the end of two hours the tubes were centrifuged and the color of the supernatant fluid of the two series compared. In the set in which the bile-salts had been added to the serum in one fraction, the first tinge of color appeared in the tube containing 0.0054 gram of the salts. The first trace of hemolysis in the tubes to which the bile-salts had been added gradually, appeared in the tube which contained 0.0050 gram of bile-salts.

A comparison of the intensities of the color in the supernatant liquid of the two sets of tubes revealed the fact that there was greater hemolysis in those tubes to which the addition of bile-salts had been gradual. In other words, the substance resulting from the gradual addition of bile-salts to serum is more hemolytic than that obtained when the addition is made all at once.

TABLE 3

0.5 PER CENT BILE-SALTS	BILE-SALTS	COLORIMETER READINGS	
		Series A, with gradual addition of bile-salts	Series B, with addition of bile salts at one time
cc.	*gram*	*mm.*	*mm.*
1.15	0.00575	12.50	15.0
1.20	0.00600	13.30	15.0
1.25	0.00625	13.80	15.0
1.30	0.00650	13.60	15.0
1.40	0.00700	13.90	15.0

The supernatant liquid of five of the corresponding tubes of the two sets were compared in a colorimeter. The results follows in table 3.

The colorimetric comparison of the two sets of tubes proves that there was greater hemolysis in the tubes to which the bile-salts had been added gradually.

CONCLUSIONS

1. The inhibitory power of the blood serum of normal persons on bile-salt hemolysis is quite constant.

2. The amount of bile-salts which fixes increasing amounts of blood serum was determined. The values conform to the equation $\frac{X^n}{C} = K$.

3. The Danysz effect was demonstrated in the bile-salt and serum system.

4. On the basis of these experiments the conclusion seems justified that the inhibitory power of blood serum on bile-salt hemolysis is due to an absorption phenomenon rather than to a chemical reaction.

Further work is in progress in which isotonic saccharose solution is substituted for physiological sodium chloride solution. Some interesting results have been obtained which will be reported in a subsequent paper.

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THE RELATION OF VITAMINS B AND E TO FERTILITY IN THE MALE RAT

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Degeneration of the rat testis as a result of vitamin E deprivation was first indicated by the work of Mattill, Carman and Clayton (1924). Their observations were made upon animals reared and maintained upon various types of milk diets.

Mattill and Carman (1923) had presented preliminary evidence secured from animals on these milk rations that the degenerative changes observed were not attributable to the lack of any of the then accepted dietary requisites. That Vitamin E deprivation is responsible for the breakdown of the rat testis has been abundantly verified with synthetic rations by Evans (1925), Mason (1925) and Mattill and Clayton (1926). To the second investigator we owe histological information upon the progress of the degeneration. The work of Beard (1926) shows that the mouse testis suffers similarly, if less promptly, when vitamin E is withheld.

The importance of an adequate supply of vitamin B for growth and reproduction has long been recognized and a relatively prompt cessation of reproduction is one of the first signs commonly observed when vitamin B is withdrawn. A degeneration of the male gonads has been considered as one of the attendant consequences of vitamin B deprivation. Allen (1919) was the first to portray the histology of the completed process. Parkes and Drummond (1925) recently made a physiological and histological study of male fertility on withdrawal of vitamin B. The degenerative changes detailed in these two studies bear so many resemblances to those found after vitamin E starvation that the query naturally arose as to the relation of the two accessories to the maintenance of reproductive capacity and to the preservation of normal gonads. A consideration of the reports of various investigators who have supplied specific information upon male fertility as affected by diet may well be postponed until the observations here to be recorded have been described.

The four synthetic rations which we have thus far employed are indicated in table 1. As is evident, they were designed to supply little or no vitamin B with and without vitamin E. Twenty-seven fully grown and, so far as was known, normal, male rats were apportioned among these four

rations. They were weighed at intervals and as facilities and available female animals made it possible they were mated for periods of six to ten days, so as to cover one and often two ovulation periods. During this time the female animals were without vitamin B but since they had abundant amounts before and after the mating period their fertility was not likely to be impaired. As the male animals began to decline they were carefully watched for symptoms of paralysis of the hind legs and were in many cases continued as long as it was felt that they would not succumb during the succeeding night. They were killed without anesthetic in order that the motility of any sperm in the epididymis should not be affected. The testes were weighed and the results expressed in terms of the percentage of the normal for the body weight as given in Donaldson's tables.

TABLE 1

RATION NUMBER	CASEIN	STARCH	LARD	COD LIVER OIL	WHEAT GERM OIL†	SALT‡	YEAST§
2	18*	68	4	2	4	4	0
3	18*	68	8	2	0	4	0
4	18	67.5	4	2	4	4	0.5
5	18	67.0	4	2	4	4	1.0

* Commercial casein slowly washed for a week with dilute acetic acid followed by distilled water.

† Extracted from wheat germ by anhydrous ether.

‡ The salt mixture of T. B. Osborne and L. B. Mendel, 1917, Journ. Biol. Chem., xxxii, 317.

§ Molasses yeast kindly supplied by the Fleischmann Company.

The pertinent data from the animals on each of the four rations are given in tables 2 and 3; the animals in table 2 were without vitamin B from the beginning of the experiment, those in table 3 were gradually deprived of it.

From a study of the testes weights and fertility of male rats on synthetic rations lacking vitamin E, Mattill and Clayton (1926) concluded that gonads less than 70 per cent of the normal weight for the body weight were rarely functional; when above 70 per cent of the normal they were generally functional.[1] Of the fifteen animals listed in table 2, only one (♂4516)

[1] Recent observations in this laboratory have shown that animals on an E-deficient diet (a lard-containing milk ration) may have testes of normal weight and appearance and yet possess only non-motile sperm in the epididymis. The sperm were in process of dissolution as indicated by a slight bulbous enlargement at the junction of head and tail and by degenerated or severed heads. Sections of the testes of these animals were not made and it is thus not possible to say what proportion of the tubules had been affected. Two of the animals were known to have been fertile two to three weeks before death by the six to eight resorptions in progress in each of the females which were examined with the males. In this connection see table 2, ♂ 93.

showed degeneration by this criterion. In all the others the decrease in weight of the testes was roughly proportional to the loss in weight of the body as a whole. This relationship Jackson (1915) found in rats during acute and chronic inanition. That the testes of these animals had not suffered degeneration is further substantiated by the presence of sperm in

TABLE 2

ANIMAL NUMBER	DIET NUMBER	TIME ON DEFICIENT RATIONS	FERTILITY DATA AFTER INTERVALS ON THE RATION	LOSS IN BODY WEIGHT USING HIGHEST WEIGHT	WEIGHT OF TESTES IN TERMS OF NORMAL FOR BODY WEIGHT	REMARKS
		days	*days*†	*per cent*	*per cent*	
132	2	55	14 (5y); 30−	43	77	Some motile sperm in epididymis
4517	2	48	22 (7y)	22	108	Sperm present in testes
4504	2	48	20 (9y)	26	112	Sperm present in testes
4516	2	48	18−	44	44	Sperm absent, marked paralysis
4514	2	48	22 (8y)	28	127	Sperm present in testes
4510	2	49	19 (8y)	22	101	Sperm present in testes
4501	2	48	18−	33	97	Sperm present in testes
93*	2	89	14 (3y); 38−	36	R 58 L 86	Right epididymis no sperm Left epididymis many motile sperm
71*	2	89	16 (7y); 38 (8y)	41	110	Epididymis contents very small but many actively motile sperm
4515	3	64	20−	36	130	Sperm present in testes
4518	3	64	19−	41	126	Sperm present, typical −B symptoms
4520	3	49	20−	28	89	Sperm present
4506	3	49	19−	31	121	Sperm present
4509	3	77	10−	49	125	
4511	3	77	19−	51	111	

* The mistaken use of unwashed casein for three weeks during the last month of life, together with a well-established habit of consuming their excreta prolonged the life of these two animals.

† Figure in parenthesis is the number of young born from the matings. A dash is a negative outcome.

the smears or of actively motile sperm in the epididymis. This was true even in the last stages of vitamin B starvation when paralysis of the hind legs and the usual gastro-intestinal symptoms (post-mortem) had appeared. In the borderline case ♂132 on ration 2 the proportion of motile sperm was reduced and in the degenerated animal ♂ 4516 the epididymyl contents

revealed only occasional sperm and these were non-motile. Of particular interest is the observation on ♂ 93 whose testes showed unequal degenera-

TABLE 3

ANIMAL NUMBER	DIET NUMBER	TIME ON DEFICIENT RATIONS	FERTILITY DATA AFTER INTERVALS ON THE RATION	LOSS IN BODY WEIGHT USING HIGHEST WEIGHT	WEIGHT OF TESTES IN TERMS OF NORMAL FOR BODY WEIGHT	REMARKS
		days	*days**	*per cent*	*per cent*	
4508	4	138	30−; 60−; 100−	43	32	Almost paralyzed, a few dead sperm in epididymis
4519	4	108				Paralyzed, many actively motile sperm in epididymis
	2	16	35−; 63 (6y); 100 (5y)	35	122	
4500	4	84	35(6y); 60−	23	131	Sperm present in testes
4502	4	84	60(9y)	20	—	Sperm present in testes
4521	4	84	35−; 60−;	20	122	Sperm present in testes
4522	4	89	35−; 60−;	19	—	Many motile sperm in epididymis
4507	5	89	40−	49	—	Paralyzed. Many motile sperm in epididymis
4503	5	119				Paralyzed, and hardly breathing; some motile sperm in epididymis
	2	38	37(6y); 100−	50	103	
4512	5	108				Many motile sperm in epididymis
	2	37	37−; 99(4y)	36	120	
4523	5	108				Paralyzed, many motile sperm in epididymis
	2	16		38	95	
4524	5	108				Paralyzed, many motile sperm in epididymis
	2	30		26	115	
4513	5	108				Many motile sperm in epididymis some hours after death
	2	27	33(7y); 100(4y)	37	112	

* Figure in parenthesis is the number of young born from the matings. A dash is a negative outcome.

tion. The corresponding epididymis reflected this difference; one contained no sperm, the other many motile sperm.

If a lack of vitamin B in the diet is apparently not a factor in testicular degeneration in the rat, why, nevertheless, do animals so deprived lose their procreative power? The functional tests carried out on these animals were very inadequate, due to limitations of space and time. The female animals used were not examined for regularity of the oestrous cycle, nor were all of them of proven fertility. In a few of the negative tests definite pathological conditions in ovary and uterus were evident, such as cysts, hematoma or pus. In cases where the mating tests were negative due to known impairment of female function, they have been omitted from the tables. Even with these uncertainities, however, it can hardly be fortuitous that none of the males deprived of both accessories gave a positive fertility test within two to three weeks after the deprivation began, while seven of the nine animals deprived of vitamin B but not of E for a similar period were still fertile and sired an average number of young. A tentative explanation for this difference is offered by the observation of Mattill and Clayton (1926, p. 677) that a temporary but marked acceleration of growth took place in animals of both sexes when wheat germ oil was added to a basal sterility-producing diet, adequate in all respects save vitamin E. This phenomenon, whatever its explanation, can not be construed as other than indicative of a higher rate of metabolism. In his monograph on human under-nutrition Benedict and his associates (1919, p. 640) and also in particular Miles (1919), express the belief, based on the reports of their subjects, that a lowered metabolism may reduce or even obliterate sex interest. There can be no doubt of a lowered metabolism in rats deprived of vitamin B, especially in view of the direct measurements made by Gulick (1924). If vitamin E tends to raise the plane of metabolism, as it apparently does, either directly or secondarily by stimulating the appetite, it is not surprising that among animals undergoing vitamin B starvation the sex instinct should persist longer in those provided with vitamin E than in those without it.

Whether or not this explanation shall prove to be valid, it is clear that male rats possessing apparently normal reproductive organs may fail to procreate and any study dealing with the relation of diet to reproduction must take into account the influence of a lowering of the plane of metabolism upon sex instinct, however this lowering of the metabolic level be produced.

Among those who have studied the appearance of testicular degeneration in rats upon diets lacking vitamin E there is very satisfying agreement to the effect that colony animals placed at weaning upon such diets do mature sexually and that complete degeneration supervenes between the 90th and 150th day of life. Older animals that have had an opportunity to accumulate a larger amount (or whose need for it is less exacting) may be deprived of vitamin E for longer periods without damage. Also since

the degenerative changes are apparently irreversible the nutritive history of an animal must be known, as well as its reproduction record before it is used for this kind of experiment. Occasionally, and for reasons not yet understood, colony animals, supposedly receiving an adequate diet, exhibit the sterility due to lack of vitamin E. Such an animal undoubtedly was the single exception in table 2.

It is of course entirely possible that a diet low in its vitamin B content, but yet not completely devoid of it should ultimately induce degeneration due to a simultaneously low vitamin E content, particularly since the food intake decreases with the progress of the animal's decline. With a view to determining the effect of a prolonged vitamin B starvation, during which vitamin E was being supplied, the animals listed in table 3 were placed upon the same synthetic ration containing 0.5 or 1.0 per cent of yeast. Finally, in order to shorten the test, they were denied even this small amount. Here again with only one exception (♂ 4508), the absence of vitamin B, even to the extent of inducing complete paralysis did not result in degenerated gonads. Motile sperm were regularly found in the epididymis, in one case even several hours after death.

The fertility data secured from these animals were likewise incomplete due to lack of control of the female animals, and negative results may not be significant. One of the animals (♂ 4519) still retained its procreative power after 100 days, another (♂ 4502) after 60 days, on a diet containing ½ per cent of yeast. This interval is much longer than Parkes and Drummond report. Two other animals (♂ 4512) (♂ 4513) were fertile after one hundred days on a 1.0 per cent yeast ration. Unfortunately the mating tests were made in cages not provided with false bottoms and the later mating periods were almost invariably accompanied by a resumption of growth on the part of the males. The weight increment during and immediately following the mating period was often very marked. This rise in the level of metabolism may possibly account for a positive result when an earlier test had been negative,[2] but the data are too meager to justify such a conclusion. Nevertheless, since the gonads were normal at death a temporary reawakening of the sex impulse might conceivably provide for a positive outcome at any time, and irrespective of previous behavior, during the deprivation period.

Obviously many of the relations just suggested require more careful study; essential in such study are the use of false-bottom cages, careful control of the mated females by vaginal smears, and separate feeding of vitamin E to the experimental males. But if the sterility arising from a deficiency of vitamin B is primarily due to a lowering of the plane of metabolism and an accompanying lowered sex expression, it is interesting

[2] Parkes and Drummond report a similar case.

to inquire to what extent the degeneration of the testes, heretofore ascribed to vitamin B starvation has been contingent upon a simultaneous or previous insufficiency of vitamin E in the diet.

The observations of Allen (1919) were made on two small lots of rats: one lot of four had been used in the Osborne and Mendel studies on vitamin B, the other lot of five animals together with controls came from MacDowell's alcohol experiments. For the former no diet data are given; they were all well developed but sterile. Presumably the B-deficient ration was a synthetic one containing butter fat 9 per cent and lard 15 per cent as was the custom in the Osborne and Mendel studies. Repeated trials in this and other laboratories have shown that whatever vitamin E the butter fat in this ration may contain, it is ineffective when such amounts of lard are present and degeneration supervenes whether B is present or not. Although recovered from the effects of vitamin B starvation, these animals still bore the marks of their shortage of E because the damage is irreparable. This conclusion is borne out by Allen's histological observations which agree with those describing vitamin E-deprived gonads. For the MacDowell alcoholized rats likewise no specific diet data are given. They were not sterile, although their fertility was reduced. Only two of the five animals had testes of subnormal weight, but of greater significance is the fact that some degeneration was observed whether the animals had been alcoholized or not. Allen comments upon the fact that similar conditions of degeneration may arise in rats subjected to widely different treatments.[3]

The degeneration exhibited by Osborne and Mendel's (1919) yeast-fed animals was obviously a result of inadequate vitamin E, since the diet contained abundant amounts of vitamin B.

The anatomical analyses of Hatai (1915) made upon some of McCollum's lipoid-free diet animals showed that the testes were 44 per cent deficient after six months. There was some stunting of body growth but the ration can not have been entirely lacking in either A or B vitamins. The specific effect upon the testes here noted constitutes an early and unrecognized demonstration of the rôle of vitamin E.

The results of Paul (1906) who fed rats on ox flesh exclusively are less favorable to the present thesis. Paul found that this diet if begun at weaning and continued for from two to six months resulted in an underdevelopment of the entire male reproductive apparatus. When adult rats were kept on it for from five to nine months the organs were slightly

[3] Kostich (1921) described more recently a reversible seminal degeneration in rats due to alcoholism; the destruction of the seminal cells proceeded in the opposite order of their generation, somewhat as Mason (1925) has outlined its progress in the absence of vitamin E; there was a very great range in the susceptibility of the animals and no diet data are given.

smaller than the controls. This food is now known to be poor in vitamin B and has been shown by Evans and Bishop (1923) to be an effective cure for the dietary sterility of female animals, but unpublished results from this laboratory show that under certain conditions a ration containing 20 per cent of cooked and dried beef muscle produces resorptions in females and degeneration in males unless additional vitamin E is supplied.

Hewer (1914) who fed thymus and milk, is clearly dealing with vitamin E deficiency, manifesting itself by degeneration as early as 70 days of age, with a histological picture agreeing in many details with that furnished by Mason (1925). Also he was unable to find any abnormality in the females although they were sterile with normal colony males. This, of course, is typical of vitamin E starvation.

The animals studied by Eckstein (1923) were on a diet similar to McCollum's rachitic diet (3143) and low in fat, vitamin A and phosphorus. The corn and wheat portion, together 66 per cent of the ration, certainly supplied more of vitamin B than of E but manifest signs of a shortage of the latter were revealed by a typical histology of the testes. Radiation did not mitigate the severity of the degeneration, as Mattill and Clayton (1926) also found. Further evidence that vitamin E was involved is found in his statement that the histology of the ovaries revealed no real functional damage but the animals were infertile when mated with normal males. Meyerstein's (1922) two male animals, one on potato meal, the other on rye meal, plainly show the influence of vitamin B upon growth, and, in his estimation, also upon testicular degeneration (2 plates). From his data the weights of the testes are 50 and 57 per cent of the expected (Donaldson). Histological description is not convincing, and no functional tests were carried out.

The recent careful observations of Parkes and Drummond (1925) on degeneration and vitamin B deficiency, when carefully analyzed, suggest that contrary to their belief, they, too, were dealing with a double deficiency, the infertility being due to the animals' loss of tone and vigor through vitamin B deprivation, the degeneration of the testes being the result of a paucity of vitamin E. Their basal B-deficient diet contained 15 per cent of butter fat which in the absence of lard should provide adequate vitamin E. That it does not do so when compounded with the rest of the ration is evident from the fact that female animals on this diet plus yeast extract did not show normal fertility. Indeed, Parkes and Drummond concluded that such a diet is deficient in a factor (vitamin X) necessary for fertility in the female, and unaware of the work of Mattill, Carman and Clayton (1924) they assumed that the male animal could dispense with it. The fact that this basal ration supplemented with 4 or 5 parts of yeast extract, while not adequate for reproduction in the female has, nevertheless, in their long experience, provided for normal

growth, nutrition and reproduction in the male, indicates that it supplies some vitamin E. But it is obviously a limiting amount which allows little or no accumulation in the tissues, since sections of the testes of their experimental animals receiving 4 per cent of yeast extract with the basal ration showed mild degeneration. If any of their experimental males were reared on this ration they were under an initial handicap, and with the diminished food intake immediately consequent upon the withdrawal of vitamin B the degenerative process only just held in abeyance was free to continue under the constantly decreasing supply of vitamin E.[4] Of even greater significance is the fact that they were able to record only one case of complete degeneration and entire absence of sperm, and they suggest that a fairly advanced stage of degeneration is not incompatible with fecundity. The prolonged maintenance of some of their animals on a meager vitamin B supply may be accounted for by the failure to use false-bottom cages, especially since yeast extract was administered daily to the mated females. Such secondary accessions of vitamin B undoubtedly explain in part the variability among their male animals in respect to loss of weight and fecundity; this variability was particularly marked on the basal ration with one per cent of yeast extract. The progress of the degeneration as they describe it is typical of vitamin E deprivation as is also the failure to restore degenerated tissue by normal feeding.

Degeneration of the testes of birds on a diet of polished rice has been established by many observations. McCarrison (1918–19) compared in pigeons the effects of inanition with those of milled autoclaved rice feeding (without and with butter, one-twelfth ounce per day) and found them very similar in that the testes decreased to less than one-tenth of their normal size. This shrinkage occurred in starved pigeons within an average of twelve days and in the polyneuritic birds it was apparently the direct result of the prolonged inanition. While spermatozoa and spermatocytes

[4] In this connection certain observations on underfeeding and refeeding as affecting the testes of rats may be pertinent. Jackson and Stewart (1919) underfed rats from birth to three or six weeks of age (from weaning time on graham bread and whole milk) and then refed on bread and milk ad lib. to a weight of 75 grams. The testes developed to little more than half of the normal weight. No other organs suffered so marked an impairment. This impairment was not noted when the underfed animals were refed to a weight of 25 or 50 grams. They suggest that possibly the earlier inanition may in some way unfavorably affect the later growth of the testes. However the testes of animals underfed beginning at weaning and refed after varying periods show, according to Siperstein (1921) merely a lag in recovery and spermatogenesis returns to normal in a short time; Stewart's (1916) limited observations on similar material point in the same direction. If underfeeding from birth has relatively more permanent effects than when it is begun at weaning and if these effects are due to a scarcity of vitamin E it becomes even more clear that for such experiments as these the entire nutritive history of an animal must be known, insofar as this is possible.

were absent, there still remained a layer of cells which from their appearance seemed capable of regeneration. It remained for Dutcher and Wilkins (1921) to show that the testes of cockerels similarly deprived of vitamin B could regenerate to some extent. Inasmuch as vitamin E degeneration in the rat is not attended by so great a shrinkage of the testes in so short a time and is unanimously considered to be irreversible, and since inanition has no such selective effect in the rat as in the pigeon a discussion of the other reports on the effects of vitamin B starvation upon testicular degeneration in birds (Funk and Douglas 1914), (Portier, 1920), (Novaro, 1921) is unprofitable until the rôle of vitamin E, if any, in avian economy has been established.

That the testicular degeneration observed in birds is of a different order from that seen in mammals is attested by the experiments of McCarrison (1919) on monkeys receiving only polished autoclaved rice for periods of 19 to 28 days. Their average loss in body weight was 26 per cent while the testes weighed more than those of the controls. From other observations McCarrison concluded that butter may contain some substance that exercises an influence on the nutrition of the testes.

SUMMARY

The alleged degeneration in the testes of rats deprived of vitamin B is so similar to that recently found in vitamin E deprived animals as to suggest that vitamin E only is concerned in the process of atrophy. This is apparently so. Of 21 male rats which succumbed under immediate or gradual vitamin B starvation, on a ration containing an adequate amount of vitamin E, only two showed degeneration when gauged by the weight of the testes or by the presence of sperm. Many of the other 19 animals were helpless from paralysis but the epididymis still contained sperm as motile as those of a normally fed animal. Of six animals deprived of both accessories none showed degeneration after an average of 63 days on the ration and after suffering an average loss in body weight of 39 per cent. Mating tests with colony females showed that this latter group lost procreative power slightly earlier than those not deprived of vitamin E although those also became sterile. This infertility in the presence of apparently normal gonads is believed to be due to the loss of tone and vigor and the consequent lowered sex expression coincident with the lowered level of metabolism. This is delayed but not prevented by vitamin E. Since an adequate supply of vitamin E is particularly necessary for the rat during the first 90 days of life the previous nutritive history of an animal is of vital importance in such studies. The mating tests were not carried out in false bottomed cages and were often accompanied and followed by marked increases in body weight. From a study of the literature establishing a relationship between vitamin B and testicular degen-

eration in the rat it appears that a dual deficiency has been involved in all these experiments. This is evident from the similar histology of the degenerated glands, from the irreversibility of the process, from the nature of the diet, and occasionally from simultaneous observations on female animals showing a behavior typical of vitamin E shortage. The reports of degeneration in birds on a polished rice diet indicate that this is of a different order from that found in mammals since it is likewise produced by starvation; there is also a much greater shrinkage of the gland and the process is to some extent reversible.

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THE EFFECT ON REPRODUCTION AND LACTATION OF DIFFERING PROPORTIONS OF MEAT IN A MIXED DIET[1]

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The purpose of this paper is to describe some preliminary experiments with rats dealing with the effect on reproduction and lactation of differing proportions of meat in a mixed diet composed of ordinary foods. Since the experiments were of one year's duration only and were intended to be of a preliminary nature, the results can be considered merely as indicative and not conclusive. For such experiments to be conclusive the number of animals studied should be much larger, each experimental animal should be continued on the diet at least through the entire reproductive life, and the diet should be studied through several generations.

Experiments were reported by Slonaker and Card (1923) in which one group of white rats was fed table scraps from which bits of meat and animal protein were removed, while another group received table scraps and in addition some form of animal protein. Better results in growth, reproduction and longevity were reported in the case of the animals receiving animal protein. No attempt was made to feed a uniform diet or to keep records of food intakes. In the experiments described here, any two groups of animals compared received diets similar in all respects with the exception of the proportion of meat in their diet, and since food records were kept in all cases, the amount of food eaten by each lot and the proportion of each constituent eaten were accurately known.

In order that the experiments should have a maximum practical value, only food materials which are found in the ordinary human dietary were used in these experiments. It was planned that in the diets containing the smaller amounts of meat, the protein should be rather low but presumably adequate for growth so that the meat added to these diets should furnish the extra protein presumably required for reproduction and lactation.

EXPERIMENTAL. The experiments were arranged in two series ac-

[1] This investigation was made possible through the award of a fellowship by the National Research Council, from the research fund granted by the National Live Stock and Meat Board.

cording as the meat was fed 1, fresh and raw with allowances of definite weights per rat per week, or 2, as a definite percentage of the dry food mixture, the beef being cut in inch cubes, dried in a large vacuum oven at 70° to 80°C. and ground in the laboratory.

The animals were kept under the conditions which have been described in other papers from this laboratory (Sherman, Rouse, Allen and Woods, 1921), (Sherman and Campbell, 1924).

First series. The dry diet used in the first series in which the meat was fed fresh and raw was as follows: whole milk powder, 12 per cent; powdered lemon juice and corn syrup (Merrell-Soule), 5 per cent; butter fat, 5 per cent; dried spinach, 2 per cent; dried carrot, 5 per cent; patent flour, 30 per cent; and starch, 41 per cent. In addition to this dry diet, some animals received 10 grams of fresh, lean beef per rat per week while others directly comparable in regard to litter and sex received 40 grams of beef per week. It was difficult to make the animals eat the liberal allowance of meat until the sixth or eighth week of the experiment in most cases.

Four lots, containing two males and three females each were first started; two received the small allowance of meat and two the liberal allowance. The animals were placed on the diet at 5 to 6 weeks of age and were continued on the diet until they were slightly more than one year of age. Three females died during the course of the experiment. Two of these received the low meat diet and died at $6\frac{1}{2}$ and $7\frac{1}{2}$ months respectively; one received the liberal meat diet and died at four months.

The adult animals ate an average of 70 grams of dry food per week. Therefore, by increasing the meat from 10 grams to 40 grams the protein consumed per week was increased from about 7.1 grams to about 13.7 grams or the percentage of protein in the food solids was increased from about 9.9 per cent to about 17.5 per cent.

The growth records of the animals on both the liberal and the low meat diets were normal.

The increase in meat appeared to make an improvement in the reproduction record in this case. If only the directly comparable females which lived until the end of the experiment are considered, a total of three females on each diet, it is found that six young were born to the females on the low meat diet and none of these were raised, whereas seventy-eight young were born to the three females on the liberal meat diet and thirty of these were raised to the standard weaning age of four weeks. The average weight of these young was 29.1 grams at weaning or below the average weight of normal animals at four weeks of age.

A male and two females from every litter born on experimental diet were continued on the diet after weaning. The animals of the second generation grew at a subnormal rate and were extremely nervous. There was considerable breeding among them but only one litter of five was

reared successfully. The animals of the second generation were unusually variable in their growth and breeding records.

Second series. In the second series, as stated earlier, the meat was dried and included in the dry diets, in one diet in a limited amount and in the other in a larger amount. The diets were of the same composition as those used in the first series except that the meat replaced a part of the starch.

The composition of the low meat diet was as follows: dried meat, 3 per cent; whole milk powder, 12 per cent: powdered lemon juice and corn syrup, 5 per cent; dried spinach, 2 per cent; dried carrot, 5 per cent; patent flour, 30 per cent; and starch, 38 per cent. The liberal meat diet contained 12 per cent of meat and 29 per cent of starch with other foods in the proportions just stated. The protein contents of these two diets were 9.1 and 14.9 per cent respectively.

Two lots, one on the low meat diet and the other on the liberal meat diet and containing two males and two females each, matched as to litter and sex, were started when the animals were 5 to 6 weeks old and two other lots containing two males and three females each were started when the animals were 3 to 4 months of age. These experiments were continued for about eleven months.

There was less breeding among the animals receiving the dried meat than among those receiving the fresh meat. Two females on the low meat diet produced eighteen young and one litter of four was raised. These four young reached the age of four weeks at the end of the experiment when the mother was slightly more than fourteen months old. The other females did not breed at all.

Of the five females on the liberal meat diet only two bred. Four young were produced by one female but all of these died. Another female produced thirty-five young of which thirty-two were raised to the age of four weeks. The average weight of these young was 35.0 grams. There was no breeding among the second generation animals on the dried meat diet. The improvement shown, therefore, as a result of increasing the proportion of meat in the dried meat diets was due to the unusually good record of one female.

Effect of increasing the milk in the diet. Although it seemed probable, calculating on the human dietary standard, that the calcium furnished by the milk and vegetables in the diets just described would be sufficient, some experiments were carried out in which one per cent of Osborne and Mendel salt mixture was substituted for one per cent of starch in the liberal meat diets. The animals taken for these experiments were directly matched as to litter and sex with animals receiving the same diet without the added salt mixture. Increased growth and reproduction undoubtedly resulted from the addition of this salt mixture.

Because of the improved results obtained when the salt mixture was added to the diets, it was decided that the best means to improve the mineral content of the diets and yet retain diets of ordinary food materials was to increase the proportion of milk in the food mixture. The milk powder was, therefore, increased to 16 per cent in the dried meat diets, the extra milk powder replacing a part of the starch. The protein contents of the two diets then became 10.2 per cent and 16.8 per cent.

Two lots, containing two males and three females each, were started at four weeks of age and continued for nearly $7\frac{1}{2}$ months, one on the liberal meat diet, the other on the low meat diet. One female in the liberal meat cage died after $5\frac{1}{2}$ months on the diet.

The increase in milk powder made no significant change in the rate of growth. The larger amount of milk in the diet made possible the successful rearing of young on the diet containing only 3 per cent of dried meat. Thirty-four young were born and twelve were raised to four weeks of age. At four weeks, the young, representing two litters, weighed from 12 to 28 grams with an average weight of 19.3 grams. One of the litters died at nine weeks of age. The other litter was six weeks old at the end of the experiment and the animals weighed less than thirty grams each.

Forty-three young were born to the three females on the diet containing 12 per cent of dried meat and 27 of these were raised to the weaning age of four weeks. The weights of these young varied from 24 grams to 46 grams with an average weight of 32.2 grams. The increase in meat in this diet, therefore, apparently had a beneficial effect on reproduction and lactation.

Although, in general, these experiments seemed to show improvement in reproduction when the proportion of meat in the diet was increased, it must be pointed out that the results are not conclusive. Nearly all of the animals were unusually nervous and somewhat below the general average of our colony in vigor. Further experiments of this type are needed to determine whether the use of other vegetables or the same vegetables in different proportions would give better results.

SUMMARY

Some very preliminary experiments have been described which were planned to study the effect on reproduction and lactation of differing proportions of meat in a diet of ordinary foods. The results of these few experiments of comparatively short duration would seem to indicate improved reproduction and lactation when the fresh meat in a mixed diet is increased from 10 grams per rat per week to 40 grams per rat per week, raising the proportion of protein consumed from about 9.9 per cent to about 17.5 per cent. When dried meat was made a part of the diet in-

stead of fresh meat, there was less breeding among the animals used in these experiments. The increase in dried meat from 3 per cent to 12 per cent, which raised the protein content of the diet from 9.1 to 14.9 per cent probably had a beneficial effect on reproduction and lactation.

When the basal diet was improved by increasing the percentage of milk from 12 per cent to 16 per cent there was successful reproduction on both 3 per cent and 12 per cent of dried meat. A greater number of young of higher weight were reared on the diet containing 12 per cent of meat.

The author is greatly indebted to Prof. H. C. Sherman for his interest and advice in this work.

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CITRIC ACID METABOLISM AS INDICATED BY URINARY EXAMINATION

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That citrus fruits are a base-forming article of diet has long been considered as established. Sherman and Sinclair (1) make the following statement: "Although mention is frequently made of the fact that some foods contain an excess of acid-forming and others of base-forming elements, no systematic quantitative study of the subject appears to have been published." Their determinations were upon calcium, magnesium, sodium, chlorine, potassium, phosphorus and sulphur. These were determined in separate portions, and their results expressed as amount of each element found to the corresponding number of cubic centimeters of a normal solution of acid or base. By adding the results of all of the bases and acids it was easy to compare the totals, and results showed the excess of acid-forming or base-forming elements in familiar terms. Blatherwick (2) in 1913 continued this work upon a number of foods, with experiments demonstrating the forecasted results by urinary examination. Sherman and Gettler (3) had, however, completed some work giving further values for foods and amplifying by experimental procedure.

Blatherwick and Long as late as 1923 make the statement that "practically every one knows that most of the various organic acids present in fruits are completely oxidized within the body." However, in a previous work they admitted that on the last day of an orange feeding experiment there was an increase of acidity in the urine. This amounted to about 6 per cent of the acid ingested. McLaughline and Blunt (4) have recently worked with fruits as a part of diet, and discussed their relation to organic acids in the urine.

Previously in an experiment upon myself, I found that eating nothing but lemons and oranges increased the urinary acidity due presumably to the failure of the body to metabolize the whole of the organic acid when thus ingested in large quantities.

As it is difficult to obtain sufficient amounts of citric acid through fruits,

[1] Last half of work was done under grant from California Fruit Growers Exchange, San Dimas, California.

and very uncomfortable to retain them on an empty stomach, chiefly because of the pharmacological action, I decided to continue this problem, using pigs as the subjects, and to determine three things: First, the amount and distribution of urinary acidity; second, the amount of citric acid excreted; and third, the relation of ketosis (acidosis) to fruit acid ingestion. Due to the fact that Blatherwick (5) had raised the question of the possibility of a flushing point or a point where the ability of the organism to oxidize the citric acid had been overstepped, I decided to try to determine this point.

The first consideration was the possibility of a tolerance, for Salant and Wise (6) reported that sodium citrate when given in large doses would produce toxic symptoms, and that second dose with the same amount or even less, would have the same result if given three days later. Robinson and Burnett (7), on the other hand, state that there was a tolerance for benzoic acid developed in feeding it to calves. This is proven because the animal returns to a normal ammonia production, even though the dose of acid was increased.

Since the excretion of acetone bodies during ketosis is due to a disturbance of oxidation power of the body, it was thought that it would most probably be affected by an increase or decrease in the oxidative power of the body.

Outline of experiment. In these experiments it was my object to determine first the reaction of the animal to the citric ion (combined with sodium) and then to determine the reaction of the animal to free organic acid, trying if possible to reach a point where the ingestion would produce an increased acidity of the urine as suggested by the previous experiment on myself, and the work of Blatherwick and Long (5). A small amount of sodium citrate was first given (weight equivalent of 100 cc. N solution) decreasing the amount of citrate and replacing it with citric acid. The dosage was increased gradually (series A), finally reaching a point where the animal produced the desired urinary acidity, and a larger amount of citric acid was excreted.

Having arrived at this so called "flushing point" the animal was returned to a normal diet for two weeks preparatory to series B, to determine if it would change its degree of tolerance, or oxidative power. While there was a high urinary acidity, citric acid excretion was very low. Acid titrations were made on 3 hour collections during this series. Later, in series E, it was again demonstrated that the oxidative power could be increased.

In the third series (series C) I tried to determine the relation between citric acid ingestion and ketosis. It was not altogether satisfactory, as we did not know to what degree a hog would produce a ketosis by starvation methods, and consequently it was impossible to judge whether or not

EXPLANATION OF THE FOLLOWING TABLES. The solutions are translated to weights as follows, because different magnifications were chosen to facilitate comparisons of the several variants:

P = 15 grams per 1000 cc.
Ca = 20.3 grams per 1000 cc.
Acetone = 58 grams per 1000 cc.
Cit. Acid = 64 grams per 1000 cc.
Ammonia = 1.4 grams per 1000 cc.

Human Series A

DAY	FEEDING*	AMOUNT OF URINE	FREE ACID	ORGANIC ACID	P	NH_3	TOTAL N	ACETONE	CITRIC ACID	Ca	REMARKS
		cc.	*cc.* $\frac{1}{10}$ *N*	*cc.* $\frac{1}{10}$ *N*	*gram*	*grams*	*grams*	*gram*	*gram*	*gram*	
1	Fast	1320	271.2	669.6	0.4385	1.667	6.765	0.0855	0.5247	0.2884	Usual laboratory work with about one mile extra walking. No hunger and only slight weakness
2	Fast	975	487.2	684.4	0.2857	0.383	6.230	0.4300	0.3939	0.3143	Weakness in morning. Little less laboratory work but same amount of walking
3	475 cc.†	1830	512.8	811.2	0.4394	0.412	5.376	0.9855	0.1772	0.3711	Felt better in afternoon after taking acid. No malaise or vertigo

* Amount given expressed in cubic centimeters of normal solution.
† 29.44 grams citric acid taken with approximately 3 liters water.

Series A (1)

DAY	FEEDING*	AMOUNT OF URINE	FREE ACID	ORGANIC ACID	P	NH_3	TOTAL N	ACETONE	CITRIC ACID	REMARKS
		cc.	*cc.* $\frac{1}{10}$ *N*	*cc.* $\frac{1}{10}$ *N*	*gram*	*grams*	*grams*	*gram*	*grams*	
1	Norm	755	301.3		0.147	0.565	4.680	0	0.0323	Hog weight 16 kilo
2	Norm	780	208.0		0.173	0.508	4.185	0	0.0803	
3	Norm	570	410.8		0.208	0.498	3.748	0	0.0799	
4	Norm	1275	388.0	592	0.392	0.615	5.888	0	0.0551	
5	N100	1560	208.0	997.0	0.348	2.721	5.167	0.1433	0.5753	N-sodium citrate fed p.m. Feeding in p.m. until further indicated. Hog weight 17.8 kilo. Water ad lib. Milk
6	Norm	1320	352.4	590.9	0.229	0.882	4.224	0.0670	0.0175	1 quart and 3½ per cent of body weight in food mix-
7	N100	970	478.4	770.8	0.1399	0.449	4.959	0.6651	0.4423	ture every 24 hours
8	N100	1575	200.0	2436	0.384	1.010	6.163	0.1910	0.6157	
9	N100	1240	288.0	2190	0.285	0.300	4.521	0.3374	1.6941	
10	N80–20	1340	195.0	1696	0.229	0.333	5.241	0.1759	0.2789	Sodium citrate and citric acid. 8–2 ratio
11	80–20	960	196.5	1375	0.155	0.410	5.132	0	0.3493	
12	80–20	500	145.0	957	0.152	0.169	4.098	0	0.22685	
13	60–40	675	164.0	1389	0.1689	0.215	5.504	0.0459	0.2415	Na cit. 60 cc. Cit. Ac. 40 cc.
14	60–40	868	140.0	1367	0.168	1.812	4.997	0.1329	0.1266	Faeces turned to more normal color. Harder, darker and with less undigested food present
15	60–40	715	136.0	1251	0.143	1.221	4.958	0.0922	0.2157	
16	120–40	1270	131.0	1944	0.298	0.620	5.538	0.0740	0.3082	Na cit. raised to 120 cc.
17	120–40	1305	86.5	2306	0.2472	0.162	5.538	0.149	0.2078	
18	120–40	1685	251.0	2287	0.364	0.208	5.967	0.0719	0.2393	
19	120–80	1530	148.0	2180	0.285	0.247	8.101	0.1374	0.2097	Cit. Ac. doubled to 80 cc.
20	120–80	1225	145.0	2169	0.300	0.393	7.032	0.1655	0.3691	
21	120–80	1090	148.0	1927	0.2343	0.2355	7.249	0.119	0.2527	Faeces granular, lighter in color and drier. Much undigested food
22	25–150	486	335.0	559	0.108	0.448	6.512	0.129	0.1241	Cit. Acid 150 cc. N solution equivalent
23	25–150	740	409.0	652	0.181	0.809	6.686	0.693	0.0606	Hog weight 23.75 kilo
24	25–150	1145	490.0	735	0.258	0.862	7.162	0.042	0.0844	

* In the column headed Feeding the quantity of sodium citrate and citric acid is indicated in cubic centimeters of normal solution. The weight equivalent was actually fed. N-sodium citrate. Sodium citrate is given first and citric acid second. Forced water intake at meals was kept constant as indicated.

Series A (2)

Forced water intake at meals was kept constant as indicated.

Series A (2)

DAY	FEEDING*	AMOUNT OF URINE	FREE ACID	ORGANIC ACID	P	NH_3	TOTAL N	ACETONE	CITRIC ACID	REMARKS
		cc.	*cc.* $\frac{1}{10}$ *N*	*cc.* $\frac{1}{10}$ *N*	*gram*	*grams*	*grams*	*gram*	*grams*	
25	50–150	1620	413	891	0.362	0.587	6.394	0.047	0.1494	Na cit. increased to 50 cc. N solution
26	50–150	745	347	887	0.187	0.858	5.614	0.149	0.21187	
27	50–150	1155	444	1142	0.254	0.798	7.720	0.1492	0.3190	
28	200	1000	597	931	0.342	0.758	7.986	0.0645	0.2078	Citric acid only from now on
29	200	1155	687	960	0.223	0.993	7.770	0.0014	0.2185	Faeces soft, dark and more in amount
30	200	1045	589	741	0.265	1.364	7.161	0.1084	0.1209	Amount of fluid increased here to 2 quarts daily with food (water 1 quart)
31	400	820	753	831	0.145	1.061	6.693	0.003	0.1383	Hog weight 27.3 kilo
32	400	750	550	689	0.163	0.820	6.114	0.043	0.1062	Increased amount of faeces, medium soft
33	400	1070	682	960	0.286	1.906	8.443	0.109	0.3566	
34	600	765	641	774	0.151	0.997	6.874	0.508	0.1095	Fed p.m. 400, a.m. 200 cc.
35	600	825	788	745	0.170	1.887	7.264	0.303	0.1108	Same feeding
36	600	675	690	796	0.159	0.910	6.829	0.224	0.1163	Same feeding
37	900	980	930	932	0.148	2.198	9.054	0.193	0.0967	Fed 500 cc. p.m. and 400 cc. a.m.
38	1100	925	787	814	0.160	0.974	7.554	0	0.0814	Fed p.m. 600 cc., a.m. 500 cc.
39	1600	1000	668	741	0.168	1.000	7.032	0	3.5617	Fed 800 cc. a.m. and p.m. Very little water consumed. Feces seem diminished in quantity
	9 p.m.	135	11.4							
	12 m.	225	12.4	Titration on 25 cc. amount, undiluted						
	9 a.m.	330	25.2							
40	2000	935	699	753	0.149	1.667	7.915	0.301	6.8978	Hog weight 31.5 kilo
	3 p.m.	230	24.55							
	12 m.	385	15.9							
	6 a.m.	100	43.6							
	9 a.m.	220	12.35							
	3 p.m.	160	20.55							
	6 p.m.	250	40.4							
	6 a.m.	185	21.4							

* In the column headed Feeding the quantity of sodium citrate and citric acid is indicated in cubic centimeters of normal solution. The weight equivalent was actually fed. N-sodium citrate. Sodium citrate is given first in the column and citric acid second. Forced water intake at meals is kept constant as indicated.

citric acid was showing a direct antiketogenic action. Series D and F on hogs were attempted to determine these things as well as series A on the human. The results will be analyzed later.

TECHNIQUE. Pigs were used in this experiment because of their easy adaptation to an acid diet. They were kept in a metabolism cage after the design of Forbes (8), which is a metal lined cage making possible the collection of urine in a bottle under the drain in the center of the cage. Urine was collected at the same time of day during each series and the cage was flushed and allowed to drain, taking care to observe the animal to make sure that no micturition occurred during the period when the cage was draining. Whatever loss of urine there was, due to failure to drain from the bottom of the cage was constant throughout.

The animal was fed twice per day and the acid, when given, was mixed with food. The weight equivalent of the number of cubic centimeters of normal solution dissolved in a constant amount of water was given. In the series where the urine was collected at three hour intervals, care was taken to see that the feeding occurred at 6 a.m. and 6 p.m. During other series the time was usually 8 a.m. and 5 p.m. The food was composed of the following:

	per cent
Corn (by weight)	50
Shorts	25
Bran	15
Tankage	10
Milk (skimmed)	1 quart

Later this was supplemented with 1 quart water in order to have sufficient liquid to mix the feed and dissolve the acid. This is indicated on the graphs. Between meals water was given ad lib.

Of the above mixture 3.5 per cent of the body weight was fed every 24 hours. The conventional figures for the above mixture are as follows:

FOOD	CALORIE VALUE	FAT	PROTEIN	CARBOHYDRATE
	therm per 100 lbs.	*per cent*	*per cent*	*per cent*
Corn	118.32	4.6	7.7	66.1
Shorts	103.73	4.3	13.48	46.2
Bran	74.95	3.0	12.5	41.6
Tankage	87.00	12.6	58.7	0
Skimmed milk	14.74	0.2	3.6	5.1

According to information given us from the animal husbandry department the above mixture should have been fed in quantities of 4 per cent of the body weight per 24 hours. This was reduced to 3.5 per cent in order to keep the hog hungry and insure complete ingestion of all food

Series B

DAY	FEEDING*	AMOUNT OF URINE	FREE ACID	ORGANIC ACID	P	NH_3	TOTAL N	ACETONE	CITRIC ACID	Ca	REMARKS
		cc.	*cc.* $\frac{1}{10}$ *N*	*cc.* $\frac{1}{10}$ *N*	*gram*	*grams*	*grams*	*gram*	*gram*	*gram*	
1	Norm	780	616	818	0.180	0.238	9.261	0.323	0.1696	0.0430	Hog weight 34.5 kilo, good health
2	Norm	705	820	818	0.141	0.952	8.720	0.121	0.0454	0.0390	N.B. Due to high acidity $MgSO_4$ was fed between
3	Norm	790	828							0.152	day 2 and 3
4	Norm	1125	228							0.110	$NaHCO_3$ given 2 days before this. 3 days lapse between day 3 and 4
5	Norm	1100	600	770	0.162	0.528	10.136	0	0.241	0.108	
6	2000	1050	911	1080	0.243	1.493	9.59	0.930	0.2275	0.1355	Citric acid given in two doses 1000 cc. a.m. and p.m.
	6 p.m.	230	13.4								
	9 p.m.	210	28.3								
	6 a.m.	485	20.0								
	9 a.m.	125	38.3								
7	2000	665	926	899	0.092	1.643	10.262	0.3573	0.4662	0.155	Same as above
	6 p.m.	215	55.6								
	6 a.m.	295	39.7								
	9 a.m.	155	25.85								
8	2000	790	661	932	0.125	1.578	9.610	0.0146	0.3314	0 120	Same as above (128 grams)
	3 p.m.	195	29.0								
	9 p.m.	255	13.5								
	3 a.m.	245	23.1								
	9 a.m.	95	31.4								
9	Fast	635	370	851	0.168	1.219	8.904	0.0337	0.1459	0.104	Fasting day to get yield of citric acid
	3 p.m.	200	29.2								
	3 a.m.	275	17.9								
	6 a.m.	60	7.7								
	12 m.	100	8.4								

* In the column headed Feeding the quantity of citric acid is indicated in cubic centimeters of normal solution. The weight equivalent was actually fed. Forced water intake at meals is kept constant as indicated.

Series C

DAY	FEEDING*	AMOUNT OF URINE	FREE ACID	ORGANIC ACID	P	NH_3	TOTAL N	ACETONE	CITRIC ACID	Ca	REMARKS
		cc.	*cc.* $\frac{1}{10}$ *N*	*cc.* $\frac{1}{10}$ *N*	*gram*	*grams*	*grams*	*gram*	*grams*	*gram*	
1	Fast	495	500	696	0.1904	0.511	8 097	0.0368	0.1430	0.078	Animal vivacious because of fasting
2	Fast	520	571	0.182	0.182	0.444	0.467	0.261	0.0203	0.079	P.M. fed 1 quart milk and 100 cc. oil. Same in a.m. with 300 cc. oil
		At 6 p.m. on the seventh animal took 300 cc. oil and milk with 51.2 gm. citric acid. At midnight, the animal developed a marked diarrhea which contaminated the urine specimen. The same mixture was offered the next morning but was refused. The animal was then given bismuth subnitrate and action was stopped. Last diarrhea at 10 p.m. on the 18th. Collection was started at 3 p.m. on the 19th. On the 18th acetone was.....						0.3427			
3	400–1600	975	160	1106	0.163	1.428	4.216	0.3417	0.0598	0.118	Animal received 200 cc. (47.6 gm. Na cit. and 800 cc. (51.2 grams) cit. acid twice per day
	6 p.m.	340	3.6								
	6 a.m.	375	8.5								
	9 a.m.	240	2.6								
4	Fast	2640	111.6	465				0.4903			Normal, no oil given
5	Fast	2615	84.0	709	0.5476	0.983	4.469	0.8565	0.0108	0.1136	Oil and agar ($1\frac{1}{2}$ per cent 500 cc.) given twice per day. No defecation for two days
6	2500	1645	256	705.6	0.448	0.645	5.723	0.1111	3.3603	0.4995	6 p.m. 64 grams citric acid (1000 cc.), 100 cc. oil and 500 cc. agar and 5 grams bran given. 6 a.m. similar food offered and about half taken. At 11 a.m. 1000 cc. N. citric acid given by stomach tube (64 grams)
	6 p.m.	560	1.0								
	6 a.m.	550	2.4								
	9 a.m.	185	10.5								
	12 m.	200	8.5								
	3 p.m.	150	7.2								

7	Fast	465	140	262	0.1739	0.415	2.1212	0.0719	0.0529	0.145
	12 m.	340	6.1							
	9 a.m.	125	17 4							
	6 p.m.	0	0							
	6 a.m.	460	19 85							
	12 m.	275	6.6							
	3 p.m.	400	3.8							

* In the column headed Feeding the quantity of sodium citrate and citric acid are indicated in cubic centimeters of normal solution. The weight equivalent was actually fed. N-sodium citrate. Sodium citrate is given first in the column and citric acid second. Forced water intake at meals is kept constant as indicated.

Series D

DAY	FEEDING	AMOUNT OF URINE	FREE ACID	ORGANIC ACID	P	NH_3	TOTAL N	ACETONE	CITRIC ACID	Ca	REMARKS
		cc.	*cc.* $\frac{1}{10}$ *N*	*cc.* $\frac{1}{10}$ *N*	*gram*	*grams*	*grams*	*gram*	*gram*	*gram*	
1	Norm	1440	239.4	529.2	0.4070	2.829	3.421	0	0.0798	0.0854	Hog weight 9.3 kilo
2	Norm	1750	225.6	517.2	0.7178	1.638	2.352	0	0.0552	0.1172	Receiving 310 grams food per day with 1 quart milk
3	Norm	2440	145.8	766.8	0.5374	1.244	2.843	0	0.8188	0.0398	
4	Norm	1085	153.6	567.0	0.4412	1.170	3.049	0	0.0470	0.0632	
5	Norm	800	117.0	460.0	0.2531	1.629	3.220	0	0.0632	0.0388	
6	Fast	785	147.0	630.0				0			Fasting started. Last feeding day previous in the a.m.
7	Fast	1240	A69	469				0.0539			A-urine alkaline to phenolphthalein expressed in cubic centimeters of 0.1 N
8	Fast	1965	A90.4	440				0.0228			
9	Fast	2500	47.0	513				0.1506			
10	Fast	1525	A148	248				0.0427			
11	Oil	2500	94.5	279				0.1608			1 liter agar and 50 cc. oil per day
12	Oil	1900	A14.4	324				0.1587			½ liter agar and 50 cc. oil per day
13	Oil	1725	A137	496				0.0259			Same feeding as above
14	Oil	2435	46	342				0.0685			
15	Oil	2000	A223	223				0.0759			
16	Oil	2450	A423	783				0.1165			
17	Oil										Fecal contamination, discarded
18	Oil	3800	Neut.	410				0.0987			
19	Oil	3365	Neut.	414				0			Urine had pH of 8.776
					Hog fasted for 11 days to produce ketosis						
31	Oil	585	38	189				0			
32	Oil	425	18.8	154				0.0248			
33	Oil	180	Neut.	165				0.0257			Animal fed 100 cc. oil per day
34	Oil	190	Neut.	115	0.0315	0.418	0.448	0.0339	0.0612	0.0832	Animal refuses water. Very weak

Specimen collected at noon and before feeding time that evening animal was given equivalent of 50 cc. normal citric acid much diluted. Animal attempted to regurgitate and died. Post-mortem examination held immediately and it was found to have a very much distended stomach.

Series E

DAY	FEEDING*	AMOUNT OF URINE	FREE ACID	ORGANIC ACID	P	NH_3	TOTAL N	ACETONE	CITRIC ACID	Ca	REMARKS
		cc.	cc. $\frac{1}{10}$ N	cc. $\frac{1}{10}$ N	gram	grams	grams	gram	gram	gram	
1	Norm	500	236	491.2	0.3229	0.414	2.352	0.0754	0.0221	0.1654	Hog weight 14.5 kilo. Food 490 grams per day plus 1 quart skim milk
2	Norm	710	315	606.0	0.2269	0.490	3.799	0.0166	0.0515	0.2016	
3	Norm	635	500	587.0	0.3559	0.840	4.197	0	0.0424	0.1616	Female animal
4	Norm	325	298	409.0	0.2799	0.641	3.813	0	0.0523	0.1391	
5	900	640	420	487.0	0.3134	1.130	4.088	0.0496	0.2095	0.1139	Citric acid equivalent of 900 cc. normal solution fed per 24 hours
	Last 120 cc. collected titrated. 25 cc.–24.5 and gave pH 5.61										
6	900	290	609	851	0.3518	0.540	1.918	0.0530	0.1177	0.1098	Water given to dissolve acid kept constant throughout experiment. Other water given ad lib.
-	6 p.m.	40	17.6								
	9 p.m.	25									
	6 a.m.	50	41.4	pH 5.66							
	9 a.m.	100	6.2	(Some diluted)							
	12 n.	75	29.8								
7	500	160	232	300	0.2421	0.270	2.139	0.0248	0.1093	0.1073	
8	500	400	377	502	0.3246	0.980	3.772	0.0158	0.1192	0.0816	
9	700	440	378	479	0.2876	0.571	3.329	0.0065	0.1215	0.0997	
10	700	410	227	528	0.3333	1.852	2.904	0.0471	0.0772	0.1995	
11	900	465	349	572	0.3360	1.666	3.369	0.0193	0.0790	0.1562	Hog weight 16.7 kilo
12	900	270	378	465	0.2360	0.326	2.582	0.0050	0.0819	0.1041	
13	1100	405	458	520	0.2471	2.941	4.079	0.1086	0.2256	0.1112	
14	1500	125	231	226	0.2500	0.546	1.462	0.0059	0.1883	0.1106	
15	Fast	210	341	431	0.2625	0.387	2.596	0.0744	0.1340	0.1219	Hog weight 18.1 kilo
											Hog repeatedly refused food containing acid so that experiment was closed†

* In the column headed Feeding the quantity of citric acid is indicated in cubic centimeters of normal solution. The weight equivalent was actually fed. Forced water intake at meals is kept constant as indicated.

† It was found in previous work that when food was refused acid given by stomach tube was regurgitated.

Series F

DAY	FEEDING*	AMOUNT OF URINE	FREE ACID	P	NH_3	TOTAL N	ACETONE	CITRIC ACID	Ca	REMARKS
		cc.	*cc.* $\frac{1}{10}$ *N*	*gram*	*grams*	*grams*	*gram*	*gram*	*gram*	
1	Fast	150	144.5				0.0482			Hog weight 13.8 kilo. Exercise 30 minutes twice per day. Water given ad lib.
2	Fast	240	149.9				0.0820			Weather very hot. Exercise same as above. Exercise 20 minutes twice per day
3	Fast	330	294.4				0.0906			
4	Fast	345	214.0				0.0987			Hog exercised once per day for 30 minutes
5	Fast	220								Same exercise as above
6	Fast	170								
7	300	200	232.0	0.1158	0.373	2.982	0.0431	0.0344	0.0754	180 grams food twice per day. 1 quart milk
8	400	285	226.6	0.1266	0.763	3.696	0.0426	0.0372	0.1296	Hog weight 14.2 kilo. Acid fed twice per day throughout series
9	500	435	233.2	0.2353	0.912	3.542	0.0184	0.1305	0.1381	
10	600	335	290.4	0.2739	1.129	4.058	0	0.1752	0.1576	
11	700	755	290.4	0.2666	1.205	4.172	0.0370	0.2040	0.1820	
12	800	420	201.2	0.2298	1.064	2.772	0.0461	0.0412	0.3014	
13	900	500	228.8	0.2564	0.862	3.881	0.0144	0.0184	0.2372	Slight fecal contamination
	Four days omitted. 1000 cc. acid fed for all but one day. Omission due to fecal contamination									
14	1000	775	440.0	0.2353	1.851	2.996	0.0250	0.2728	0.2457	
15	Fast	675	66.0	0.2469	1.786	3.332	0.0622	0.0139	0.1355	Sample shows hematuria, from injury
16	Fast	450	57.2	0.2469	0.921	1.848	0.0661	0.0185	0.1313	Exercise 30 minutes twice per day, same for second day
17	Fast	190	49.5	0.1941	0.162	2.394	0.0253	0.0423	0.0593	Exercise a.m. 30 minutes. p.m. 20 minutes. During exercise when given, animal was brought to the point of complete exhaustion. Hog weight 15.4 kilo at close of experiment

* In the column headed Feeding the quantity of citric acid is indicated in cubic centimeters of normal solution. The weight equivalent was actually fed. Forced water intake at meals is kept constant.

offered, no matter how heavily loaded with acid. The hog, however seemed to gain weight at a fairly even rate in each instance. During series C the hog received oil (Mazola corn oil) and milk before diarrhea occurred, and oil and agar, and plain agar afterwards. During series D the hog received agar with the oil the agar being given to prevent diarrhea.

The urine was preserved by adding a few drops of toluol to the clean bottle each day before starting the collection. It was measured and made up to the next half liter. Folin's method of total acid titration was used in the presence of oxalate (9). The organic acids present both free and as salts are estimated by the Van Slyke and Palmer method (10) used by Blatherwick in his recent work on *Orange Metabolism*, and also by McLaughline and Blunt in their work. The authors of this method claim that it is accurate for 95 to 100 per cent of the organic acids present. It included, however, creatinine and creatine, and they admit that there is a possibility of unknown acids and the salts of weak bases being titrated in this method.

Phosphorus was determined by the method of Bell and Doisy (11). Ammonia was determined by Folin's Permutit method (12), total nitrogen by the Folin-Wright Simplified Macro-Kjeldahl method (13), acetone by the Van Slyke method (14), and citric acid by the method of Amberg and McClure (15) with the later modification of McClure (16). Calcium determination was added in series B and carried throughout the rest of the experiment, and was done by the McCrudden method (17).

Discussion. There seems to be a definite relation between the total amount of free acid excreted in 24 hours and the total production of urine, which extends further than can be explained by dilution. L. J. Henderson and Palmer (27) found that there was a relationship between quantity of urine and acidity, stating that when the quantity increased the free acidity decreased and vice versa. When the sodium ion was given a diuretic action was expected. On the third day of series A, the diuretic action is seen, also on the 18th day when the amount given was doubled. The reverse picture was demonstrated on the first day that citric acid alone was fed. (Series A.) This effect is noticed in series B and E; and in series C it is most striking, for with an enormous volume (2615 cc.) on the fasting day, it dropped to a smaller volume (1655 cc.) on the day acid was administered. Data taken from titrations on the three hour collection basis, show this very markedly, as there was a period of approximately 21 hours (between 9 a.m. on the 7th day and 6 a.m. on the 8th day) when the hog did not micturate. Besides the fluid given with meals, water was given ad lib during the entire experiment. Of course in series C and F there was a decrease of the amount of forced fluid intake because of the absence of milk at meal times.

A very interesting fact was found from the three hour determinations, which is that the animal gave a fair degree of regularity of micturating in the three hour period before each feeding, between 9 and 12 hours after the last citric acid food. If there was any excretion between these collections, it was usually of a lower acidity than these. Especially was this true for the first period after feedings, which showed this decrease due to the well-known alkaline wave following digestion.

The highest acidity recorded for any single specimen was in series B, at 6 p.m., collected on the second day of acid feeding in connection with the extremely large dosage of citric acid. The 25 cc. sample titrated 55.6 cc. 0.1 N alkali. A pH reading was not obtained on this material, but it was obtained on similarly high acid titrations recorded on three hour collection specimens in series E and at no time did it become lower than 5.61. Accompanying this high acidity for this one period was the highest acidity for the 24 hour specimen, and the highest excretion of ammonia for series B, showing that the total excretion of acid was at its highest. Hence it is definitely shown that organic acid could produce an increase in urinary acidity. The substance responsible for this acidity is as yet undetermined. There was a decrease in phosphorus excretion, only a slight increased withdrawal of calcium from the blood stream, a decreased amount of organic acidity, and only a very slight increase in citric acid excretion. (Examination of graphs in series B show these results.)

As regards the literature references on the subjects, Blatherwick and associates have published various articles, and he states that after eating oranges and raisins a high pH (low H ion concentration) was found with a high ammonia output and a high uric acid excretion (2). He later found that there was a decreased pH after eating prunes and cranberries (4), and in another investigation (5) about the same time he discovered a low pH on the last day of an orange feeding experiment. In regard to the results obtained with acid feeding on the fasting days, Fiske and Sokhey (18) make this statement: "A fasting basis does away with increased acid excretion. The titratable acidity is nearly at its limit any way, and acid feeding does not have much effect unless the buffer content is increased." The only instance in which my experiment agrees with this observation is in series F during the first five days fasting when the heavy exercise and warm weather was holding the total volume of urine to an exceptionally low figure.

Calcium excretion was added as a determination in series B in order to try to gain some information as to the cause of increased acidity, as there was a possibility that this was due to a withdrawal of base from the urine, rather than to an increase of acid. The data from this determination, however, show too little calcium present at any time to have an appre-

ciable effect. It is probable that the calcium in the food was sufficient to prevent a call on the tissue alkali reserve, and there was no risk whatever of a decalcification of the bones, which has been suggested by Steenbock (19) as the last means of emergency supply. During the fasting period of series C the slight increase came from the tissue reserve. Lamb and Evvard (20) show that in sulphuric acid feeding when the animal was in negative calcium balance, there was not an appreciable amount of calcium used to neutralize the ingested acid,—the effect on calcium was negligible in either direction. Their conclusions have been substantiated by Mendel and Givens (21), and McCollum and Davis (22). Lamb and Evvard further state that if the animal had been on low calcium ratio for some time then variations in excretion might be significant. A great porportion of the acid in the blood and tissues is probably buffered by the proteinates.

Phosphorus was found to be unimportant with reference to the fluctuations of the total acidity of the urine, for in the first series (series A) when the free acidity decreased the phosphorus excretion was somewhat high, and when the acidity began to rise with heavy acid ingestion the phosphorus excretion started to gradually decrease. The changes were not sufficient to have any very marked influence on the titrations. Although there is a considerable percent of fluctuation, the total amount excreted at any one time during the entire experiment is never large.

In this experiment ammonia seems to be the first defence of the body against unoxidized acid. In general a rise of acid is accompanied by a rise of ammonia excretion. However there are two marked exceptions, both being when sodium citrate was fed,—the first upon the first day of sodium citrate feeding,[2]—the second at the time of the second proportional increase of acid over citrate (series A, day 14). In the second it appears to be a defence of the body against increased acid in the blood-stream, as the body oxidative power and the alkali reserve have become adjusted to the ingestion of a certain level of acid radicle, offset by sodium ions, and when the latter was removed, the acid was left to be compensated for by ammonia production. In the latter half of series A and in series B and E the acid and ammonia increase together, although by no means proportionally. In series C, when the sodium citrate and a large quantity of citric acid were fed there was a marked increase in ammonia production, even though there was a continued drop in acid excretion. This might be explained as the first line of defense against acidulation. At the time

[2] Experimental error may possibly have crept in here, as it seems doubtful a priori that ammonia would constitute one-half of the total nitrogen, and it was not possible to verify this figure by a repeat The unknown and the standard were of such different concentration in this one case that the color comparison was approximate rather than precise. This source of inaccuracy was avoided in all later tests.

of the addition of pure acid later in this series, the oxidative power had been so stimulated that the animal was able to cope with and oxidize the ingested citric ions. Sherman (3) found that there was an increase of ammonia from acid forming foods, due to organic acids and phosphoric acid. Blatherwick found that oranges produced an increase of ammonia (2), and there is no doubt that feeding in series A of my experiment was responsible for the failure of free acid excretion to increase. This may also have been a limiting factor in the last part of series E.

Gamble *et al* state that urea nitrogen is about 70 per cent and ammonia nitrogen 10 per cent of the total nitrogen. Lamb and Evvard (28) however obtained higher percentages (up to 35 per cent) in their experimental work with sulphuric acid feeding to hogs, and also state that there is a great variation found in hot weather due to the water intake,—days with high water intake giving a higher ammonia excretion. In my experiment there seems to be a general increased amount of nitrogen excreted to correspond to the increased ammonia excretion. As the animal was a young animal the increase in nitrogen due to increase in size must be taken into consideration.

The method of Van Slyke and Palmer for organic acid titration was used so as to have comparative data with the experiments of McLaughline and Blunt (4) and of Blatherwick. As to the significance of the method there seems to be some doubt. Because of the many interfering substances in urine, and because of the wide variation in end point, it was considered in this experiment to be inherently unprecise. While the organic acid titration may run parallel to one or another of the factors present, both the acetone, citric acid and ammonia may drop down to a low value and still this organic acid titration remain high, much higher than the value of the total titratable acid. This much of importance may be given it,—the fact that it seems to give some indication of the oxidative power of the body. However to use it as a determination of the amount of organic acid radicles recovered through urinary excretion seems to be far from accurate. The difference between the value of organic acid titration and that of citric acid and acetone determination would lead one to believe that the undetermined acid is probably some intermediary product in the metabolism of citric acid, for as the body seems to gain in oxidative power (at the end of the second big rise in the organic acid curve—series A) the organic acid titration value drops, and remains low through the rest of the series. This fact, together with the low citric acid excretion, would indicate a very great tolerance, which Blatherwick mentioned, and which he considered an oxidative function of the body (29).

In series B, the organic acid for the normal days was about the same level as that at which it finished in the preceding experiment a week previously on the same animal. In this experiment it was found that a

slight rise in organic acid value was accompanied by an equal rise of ammonia and organic acid on the day when sodium citrate and citric acid mixture was fed, and on the second day of the fast there was almost exact agreement. Series E shows parallel relations only between organic acid and free acid.

Exactly as has been found in man by Amberg and McClure (15) and Amberg and Maver (24) the pig has a normal urinary constituent that gives all of the tests for citric acid. The normal excretion in our hogs (weighing from 9 to 30 kilo) is a small fraction of a gram. Any degree of citric acid feeding that raised the citric acid output to several times the normal excretion, was looked upon as above the flushing point. Series A and B show plainly that the flushing point does not correspond to a definite level of intake, but is dependent upon the oxidizing ability of the animal at any one time. On a smaller scale series E proves the same thing. The point, however, to be emphasized is that citric acid will come through in an appreciably larger amount if the feeding at any certain time is sufficiently large and the conditions favorable. Even at this time we must not lose sight of the extremely large amount which the animal was able to handle, as the dosage in the final stage in series E is almost one and one-half times the amount per kilo of that on the fortieth day of series A. This is conclusive proof that the oxidative power of the animal was materially increased. In series A it would seem that the power of oxidation had not been able to increase as rapidly as the dosage was increased, and in series C it might be that this power had retrogressed (although series B did not show this) or that the acids present in greater amount in the body because of fasting had usurped some of the oxidative ability of the animal, rendering it incapable of handling the ingested citric acid as efficiently as it had previously in series B, where we had practically no excretion of critic acid (hardly above normal), and a slight reduction of acetone bodies present. It was also found that the rest of two weeks between series A and B produced practically no retrogression of the oxidative power of the animal. Evidently it would have taken a larger dose of citric acid to have produced a flushing point in the second series, and to obtain the same picture as that which was obtained at the close of series A. In the two young animals (series E and F) it seems to be even more difficult to reach a point where citric acid will be excreted in an appreciably increased amount, although if we consider the per volume acidity of the urine alone, on day 6, series E, and on days 12, 13, 14 and 15 of the same series, we have reached the flushing point.

It is quite evident, therefore, that the animal developed a very remarkable power of oxidation. And indeed we gathered the impression from series C that the failure of the animal to produce a ketosis and the immediate reduction of the small amount produced was due to the increased

oxidative power of the animal. However further experimentation was necessary to determine the susceptibility of a pig to ketosis from starvation (or exclusive fat feeding), and to test whether or not citric acid shows any direct antiketogenic influence such as Shaffer found glucose to possess. Series D represents a futile attempt to produce a ketosis on a 9 kilo hog by starvation supplemented later by a limited diet of mazola oil and agar. Although this treatment ran for 29 days, at which point the animal succumbed, there was never more than 160 mgm. total acetone bodies excreted in a day. This occurred on the first day of fat feeding (day 11) and the analyses for later days are very much smaller still. A further attempt to produce ketosis is recorded in series F, where starvation was combined with strenuous exercise in 30-minute periods twice per day. However at no time did this hog's total acetone bodies exceed 98 mgm. After gradually increasing the amount of citric acid ingested from 300 to 1000 cc. N solution and maintaining this amount for four days the animal was again fasted and exercised for three days similarly to the first days of this series. The total acetone bodies for any of the last three days never exceeded 66 mgm. Through-out the entire series (F) the comparative failure to produce a ketosis would prevent one from drawing definite conclusions as to the effect of the heightened oxidative power of this animal toward the production of acetone bodies. The experimental conclusions show that citric acid is not antiketogenic in the sense that carbohydrates are, but on the other hand the transitory ketosis for which it seems some times to be responsible, disappears with 36 hours despite continuous high feedings, showing that it is by no means in the class of ketogenic substances in the ordinary sense. The fact that the body adjusts itself to take care of this changing dosage given the animal, might lead one to theorize that the citric acid demanding the oxidation power of the body on these days, preventing the carbohydrates from becoming fully oxidized, and hence according to Shaffer's theory (25), there was not the necessary number of antiketogenic molecules, thus the production of a ketosis. It is of course granted that acetone does not occur in the blood, but only its precursors, which may be other intermediary products besides oxy-butyric and aceto-acetic acids.

As to the direct antiketogenic action of citric acid, evidence afforded in series F in conjunction with the three day fasting experiment (series A human) certainly points toward a negative conclusion. However, conditions prevented sufficient data to be fully convincing. It should be noted that on the third day of the fast, although citric acid was ingested (29 grams) there was no apparent check on the steady increase of total acetone bodies excreted. This difference between citric acid and glucose surprised us, because Greenwald (30) had found that injection of the

citric radicle produced glucose in diabetes mellitus and in phlorizinized dogs, and established that citric acid can be converted into glucose.

It must be admitted that the oxidative power of the hog was enormous to handle as much as 4 grams per kilo in series B and E without excreting more than 0.5 gram of citric acid. Only about 0.4 per cent of the acid ingested failed to undergo at least a partial oxidation. Considering this with the other experimental findings it might not be amiss to suggest tentatively the possibility that if the tests should be carried out upon an animal that is, like man easily susceptible to ketosis, this oxidative power might be carried over to act upon the acetone precursors in a way to limit their excretion.

CONCLUSIONS

1. The animal under cumulative citric acid feeding developed a remarkable power of oxidation, as is shown by the decrease of organic acid excretion and low excretion of citric acid.

2. A pig has a low liability to ketosis. The small amount produced is not sufficient to show a conspicuous decrease in acetone bodies produced because of this increased oxidative power.

3. There is found to be a flushing point where the body can not cope with the quantity of acid ingested, causing an increased excretion (this does not mean mass excretion) of citric acid itself. This is only a relative point, varying as the conditions of the experiment vary.

4. There is found to be a definite relation between the total amount of free acid excreted in 24 hours and the total production of urine. As the former increases the latter decreases.

5. There seems to be no evidence of any appreciable importance attached to the calcium excretion in the compensation against excess of citric acid intake.

6. Phosphorus was found to be unimportant in the increased acidity of the urine caused by citric acid ingestion.

7. Total organic acid titration shows no direct relation to citric acid or acetone excreted, but does give some indications of the changes of oxidative power of the body, by revealing the escape at times of unknown partial oxidation products of the citric acid.

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VITAMIN B IN COMMERCIAL MEAT RESIDUE AND COMMERCIAL EGG ALBUMEN

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In the course of our work feeding artificial mixtures of isolated food substances to dogs (Cowgill, 1923) it proved desirable to feed several mixtures differing with respect to protein or other foodstuffs but alike in this respect that all were relatively free from vitamin B. Commercial meat residue[1] and commercial egg albumen were possible sources of protein available in large quantities, which seemed satisfactory for the particular problem being studied, but the vitamin B contents of these products were not known.

That muscle contains *some* of the antineuritic factor has been shown by several investigators studying the polyneuritis induced in birds by a diet of white rice (Grijns, 1901; Eijkman, 1897, 1906; Holst, 1907; Cooper, 1912, 1913), as well as by Osborne and Mendel (1917) who experimented with rats. The last named investigators examined meat powder (dried whole muscle), meat extract and meat residue. The amount of vitamin B present in each of these products proved to be extremely low. In their diets the meat residue constituted not over 18.5 per cent of the total mixture. These findings together with consideration of the solubilities of vitamin B and its chemical stability under various conditions, and regard for the treatment which muscle receives in preparation of the commercial extract suggested that commercial meat residue would be found to have little, if any, of the antineuritic vitamin. Simmonet (1920) apparently determined that meat residue lacks vitamin B for this material was employed successfully as a source of protein in vitamin B deficient diets used with pigeons. The experiments reported in this paper show that this material is indeed lacking in the antineuritic factor. *Commercial meat residue,* when fed so as to constitute as much as 44 per cent of a food mixture having a caloric value of approximately 5.2 calories per gram, *is markedly deficient in vitamin B.*

The vitamin B content of *egg white* does not seem to have been investi-

[1] By this term is meant the insoluble residue obtained in the manufacture of commercial beef extract. The particular product used in these experiments was obtained from the Valentine Meat Juice Company, Richmond, Virginia.

gated, so far as can be judged by examination of the literature. Chick and Hume (1917) in their study of the effect of high temperatures on this vitamin experimented with *whole egg* and found vitamin B to be present. Their physiological tests were made on pigeons. Hoagland and Lee (1924) found the antineuritic value of hen's egg to be low. Numerous investigators (Eijkman, 1911; Cooper, 1912, 1914; McCollum and Davis, 1915; Steenbock, 1917; Osborne and Mendel, 1919) have observed the efficacy of egg yolk in protecting against or alleviating the symptoms of vitamin B deficiency in the pigeon and in the rat. Apparently egg white alone however has not been tested previously.

Inasmuch as the preparation of commercial egg albumen (scales) may involve inaccurate separation of the white from the yolk, the presence

TABLE 1

	DIET M R—I	DIET M R—II
	per cent	*per cent*
Meat residue	22	44
Starch	47	25
Lard	18	18
Butter fat	9	9
Salt mixture (Osborne and Mendel, 1917)	4	4

	DIET E A—I
	per cent
Egg albumen, commercial, cooked	35
Starch, corn	34
Lard	18
Butter fat	9
Salt mixture (Osborne and Mendel, 1917)	4

of vitamin B in the product is a possibility. It was decided to submit this material to the test of experiment, the result of which is presented in this paper. *Cooked, dried, commercial egg albumen,* when fed so as to constitute 35 per cent of a food mixture having a caloric value of approximately 5.2 calories per gram, *is markedly deficient in vitamin B.*

Tests with rations containing more than 35 per cent of this material were not made because experiments with dogs had shown that feeding this amount of egg albumen resulted in elimination of rather soft ill-smelling feces, indicative of intense intestinal putrefaction. Changing the diet of the dogs to one having casein or meat residue as the protein changed the character of the stools. Placing the animals again on the egg albumen ration resulted again in production of highly putrefactive stools. It was sufficient for our purpose to determine whether or not the egg albumen in the amount here used was a carrier of appreciable amounts of vitamin B.

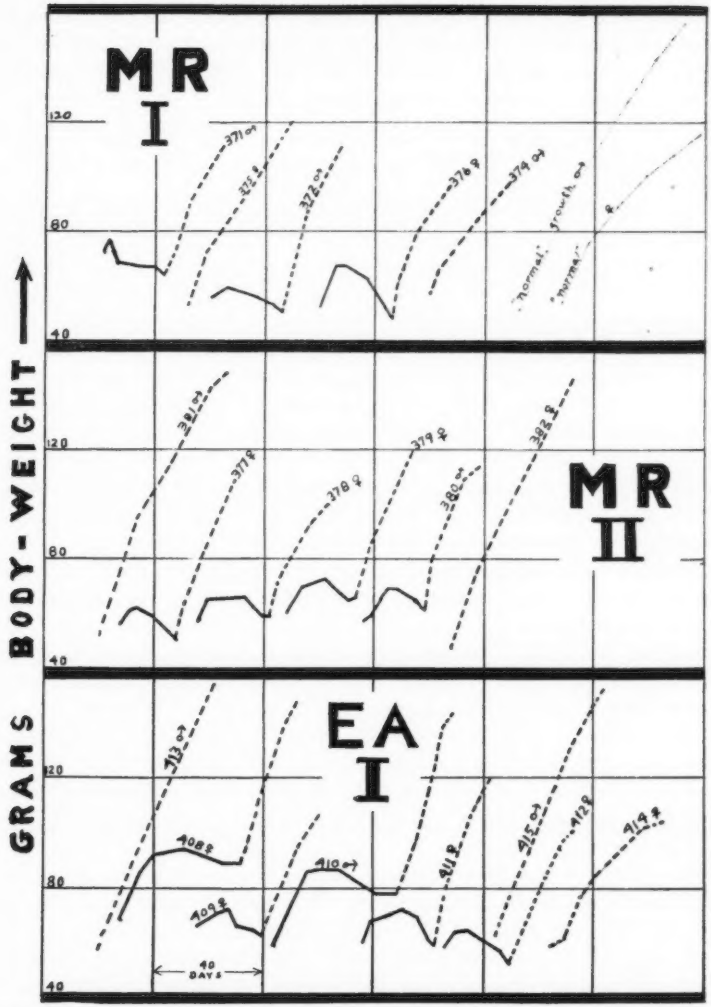

Chart 1. Solid line indicates test diet without vitamin B supplement. Broken line indicates test diet plus yeast extract.

EXPERIMENTAL DETAILS. Young white rats, each weighing approximately 60 grams, were used as test animals. Each rat was caged separately in accordance with the technic employed in this laboratory. False-bottom cages were not used, these experiments having been performed

during the winter of 1922–23, before the importance of preventing the experimental animals eating their feces and thus conserving their vitamin B supply was appreciated (Steenbock, Sell and Nelson, 1923). Inasmuch as the rations tested under our conditions proved to be quite free from the vitamin, any criticism based on failure to guard against ingestion of excreta seems of no consequence.

The diets used are given in table 1.

The commercial egg albumen[2] was found by preliminary tests to consist almost entirely of uncoagulated protein. In view of Bateman's (1916) observation that uncoagulated egg white is not well tolerated either by man or animals, when fed in moderate to large amounts, the commercial material was cooked before being incorporated in the test ration. The scales were first dissolved in water to give a concentrated solution having the physical properties of a thick syrup. This was slowly poured into a large volume of water having a temperature of 90–100°C. Adjustment of the reaction to the optimum for coagulation was accomplished by addition of 4 N acetic acid. When coagulation was complete, the curd was allowed to drain on cheese cloth, squeezed in a press to further remove water, comminuted by being passed through a meat grinder and then spread out and allowed to dry in a current of warm air (70–90°C.). Many samples have been made in this way and they have quite uniformly analysed to give approximately 13 per cent of nitrogen corresponding to 81 per cent protein.

Some of the animals received the test diet without vitamin B supplement until failure to grow and a marked decline in body weight had occurred, after which they were given daily doses of 30 milligrams of yeast vitamine powder (Harris), lot 985.[3] Administration of the vitamin in every case was followed by the characteristic prompt resumption of growth. Control animals received the same diet plus the vitamin supplement from the beginning of the feeding trial and showed growth that was normal or better.

The growth curves of the experimental animals are shown in chart 1 and require no detailed description. It might be remarked that rats 408 and 410—see group E A I—had received liberal amounts of dried yeast daily during a preliminary period because they were considered too small to start on a vitamin B-free diet. Apparently these animals stored some of the dietary essential. The longer period of satisfactory growth which these rats showed before giving evidence of vitamin lack is more likely to have been due to utilization of the stored vitamin than to

[2] The material used in these tests was obtained from the Arthur H. Thomas Company, Philadelphia, Pennsylvania. It can be obtained usually from bakery supply houses.

[3] From the Harris Laboratories, Tuckahoe, New York.

the presence of this factor in the ration. The behavior of the other animals in the group supports this interpretation.

SUMMARY

Commercial meat residue—the insoluble residue obtained in the manufacture of commercial beef extract—when fed so as to constitute 44 per cent of a food mixture having a caloric value of approximately 5.2 calories per gram, is markedly deficient in vitamin B.

Cooked, commercial egg albumen, when fed so as to constitute 35 per cent of a food mixture having a caloric value of approximately 5.2 calories per gram, is markedly deficient in vitamin B.

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A SPECTROPHOTOMETRIC STUDY OF BLOOD SOLUTIONS

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The quantitative relationship between the absorption of light and the concentration of a hemoglobin solution, has been subjected to experimental test by many investigators ever since K. Vierordt (1873), (1876) pointed out the value of spectrophotometry in biological work. The factor which expresses this quantitative relationship is usually designated as the absorption ratio (A), which is a constant for a particular substance and at a given wave-length. The determination of the value of this constant for hemoglobin depends, not only upon a spectrophotometric method of measurement, but also upon some other method which determines the concentration of the solution used. Table 1 gives the value of the absorption ratio (A) obtained by a number of investigators, and the spectral region at which the measurements were made.

The very valuable and comprehensive work of P. Hári (1917), (1919) includes a critical review of this older work, therefore repetition here is unnecessary. However, most of these data that have been accumulated, is based upon spectrophotometric absorption measurements made upon solutions of weighed amounts of crystallized oxyhemoglobin in a definite volume of solvent.[1] Because the oxygen capacity method as outlined by Van Slyke and his co-workers (1924) has proven to be so eminently satisfactory for the quantitative analysis of hemoglobin, it seemed desirable to repeat previous work, and use as a basis for the optical constants, an analysis of the oxygen capacity, rather than using weighed amounts of moist crystals where the water content might be a questionable factor.

EXPERIMENTAL. *The determination of concentration of hemoglobin in the solutions used for spectrophotometric examination.* Venous blood taken

[1] The work of Newcomer included measurements of the oxygen capacity, but his color analyses were done with a quartz spectrograph, which, because of low dispersion, could hardly give reliable figures for the visual area. Butterfield's experiments included iron analyses as well as measurements of "carbon monoxide capacity" as described by Hüfner.

from dogs was either citrated or defibrinated and then subjected to complete aeration by rotating small amounts in a large cylinder that was open to the air. The measurement of the oxygen capacity was done in strict accordance with the method as outlined by Van Slyke. Two types of apparatus were used: One measuring the O_2 under constant pressure and the other measuring the O_2 at constant volume. At least 4 or 5 analyses were made on each type of apparatus from every sample of blood. The values obtained for the combined oxygen on each blood sample were averaged, and the deviation from the mean calculated. The mean volume per cent of O_2 was transposed into an expression of grams hemoglobin per

TABLE 1

Absorption ratio (A) for the maximum in the green band

AUTHOR	YEAR	SPECTRAL REGION	$A \times 10^{-3}$
		$m\mu$	
Hüfner	1877		1.15
v. Noorden	1880	547–535	1.00
Otto	1882	547–535	1.01
Otto	1885	547–535	1.40* / 1.08†
Krüger	1888	552–536	1.30
St. Martin	1898	549–538	1.33
Cherbuliez	1890	541–536	1.326
Hüfner	1894	542–531	1.31
Aron and Müller	1906	546–534	1.25–1.46
Bardachzi	1906	542–531	1.33
Butterfield	1909	542–535	1.195
Letsche	1911	543–534	1.32
Hári	1917	541–533	1.168
Williamson	1916	542–534	1.179
Newcomer	1919	543–539	1.14

* Hüfner Apparatus.

† Vierordt Apparatus.

100 cc. of blood, by multiplying by the factor 0.746. It was considered desirable to express the concentration of the hemoglobin solutions used in the spectrophotometric part, as grams per 100 cc. rather than volume per 100 cc. of combined oxygen. The results were used in the spectrophotometric part wherever concentration entered as a factor in the calculations. Duplicate determinations of the oxygen capacity showed on the average a maximum deviation from the mean of 1.5 per cent. This accuracy is about the same as that which can be expected from color measurements on a spectrophotometer. Tabulation of this part of the data is considered unnecessary.

The spectrophotometer. The instrument used is a Bausch and Lomb

spectrophotometer. It includes a spectrometer with an Abbe constant deviation prism of $N_D = 1.72$ and also a Martens photometer mounted on a suitable optical bench. The light source is a concentrated filament 250 watt lamp mounted at the end of the optical bench, and completely housed except for a window of double ground glass. The photometer is mounted on the opposite end and between the light source and the photometer are holders for specimen tubes. A double lens and prism system produces parallel beams of light focusing on the aperture of the photometer. The photometer is of the polarization type in which the two entering beams are polarized at right angles to each other by means of a Wollaston prism. Variation in the intensity of the light between the two halves of the photometer field is made by rotating an analysing nicol prism of the Glan-Thompson type. Readings are taken from a circle divided into degrees and tenths. The light enters the photometer through 5 mm. apertures which are 40 mm. apart. This separation is greater than usual and is made possible by using a biprism with another biprism cemented to the Wollaston prism. On account of slight polarization at the surface of these prisms, the match point of a Martens photometer is never exactly 45°. The extinction points are, however, accurately set at 0° and 90°. The method employed in calculating absorption is independent of the match point so that an exact setting at 45° is unnecessary.

Calibration of the spectrophotometer. The prism of the spectrophotometer is mounted on a table which may be rotated by a micrometer screw. The degree of rotation is measured on a drum with a wavelength scale reading in mμ. The scale was carefully calibrated by the makers, but was further checked as follows: The spectrometer was placed before a mercury arc light and adjusted so that the spectral lines were sharply in focus. The wavelength drum was then turned to the graduation corresponding to 546 mμ. At this point the ocular slit was centered on the green Hg line. The ocular slit so adjusted was then kept in this position. By turning the prism table, the other lines of the spectrum could be brought to the center of the ocular and the reading on the scale compared with the known wavelength for Hg lines. Sodium and lithium flames also were used and table 2 gives the drum readings observed.

The collimator slit is of the unilateral type and the width of the opening is controlled by a micrometer screw with a head reading to 0.1 mm. In order to estimate the purity of spectrum resulting with various widths of collimator slit, several spectral lines were examined. Table 3 gives the extent to which spectral lines are broadened by opening the collimator slit. In the spectrophotometric analysis of blood solutions included in this paper, the collimator slit width never exceeded 0.05 mm. except in regions below 500 mμ. Impurity of spectrum was therefore considered negligible. Several authors have advocated working with constant slit widths and

apparently without regard to the intensity of the observation field. Certainly, a most important factor in subjective photometry is the constancy of the brightness of the field. According to Fechner's law, the minimal difference which can be distinguished in the intensity of adjacent fields, is proportional to the total intensity or,

$$\frac{dI_m}{I} = K$$

K is about 1 to 2 per cent under ideal conditions. Consequently, in this experimental work on absorption measurements, the collimator slit width

TABLE 2

	LINES	SCALE READINGS
	$m\mu$	$m\mu$
Hg	435.8	436
Hg	546.0	546*
Hg	576.9	577
Hg	579.0	579
Na	589.0	589
Na	589.6	589.5
Li	670.7	671

* Coincident setting made.

TABLE 3

Width of spectral lines in mμ

SLIT WIDTH	Hg 436 mμ	Hg 546 mμ	Hg 577 mμ	Hg 623 mμ
mm.				
0.02	Entire emission spectrum appeared as sharp lines			
0.05	0.2	0.6	1.0	1.2
0.10	0.6	1.7	1.9	2.1

was varied at random, keeping the observation field always at minimal brightness, in order to detect the smallest difference in intensity.

The eyepiece of the spectrophotometer is provided with a shutter which serves to limit the region of the spectrum to be observed. It opens from the mid-line symmetrically and is provided with an arbitrary scale. Before attempting any absorption measurement, it is important to ascertain the width of spectrum under observation. Obviously, on account of difference in dispersion, for any particular setting of the shutter, the width of spectrum included will be smaller in the blue region than the red. Calibration was carried out as follows: The ocular shutter was opened to various widths corresponding to 0.5, 1.0 and 2.0 divisions on the arbitrary

scale. The lines of the Hg arc were viewed through these openings and a record made of the difference in mμ (read on wavelength drum), when a particular line was moved from one edge of the shutter to the other. The results in figure 1 give the required data.

In the following absorption measurements of hemoglobin, the shutter was set at 0.5; then at 540 mμ, the region of maximum absorption in the green, the observation field extended a little over 3 mμ. At a wavelength setting of 540 mμ this opening would be actually 538.5 mμ to 541.5 mμ. In the region of the Na line, this same shutter width would include about 5.0 mμ. The effect of a change in ocular shutter width on absorption of hemoglobin was tested as follows: A sample of blood was diluted 1:100 with 0.4 per cent ammonia, placed in a 1 cm. cell, and examined in the spectrophotometer at 540 mμ (mean) and at 575 mμ (mean). Absorption measurements were made on the same sample using different ocular shutter widths.

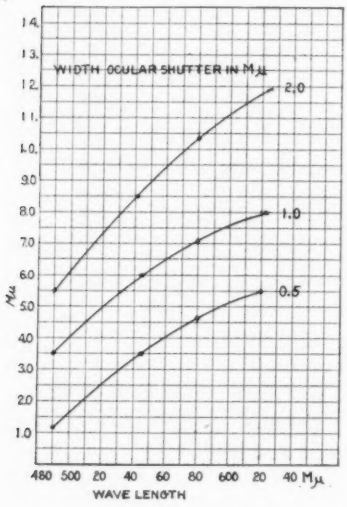

Fig. 1

It can be seen from table 4 that the extent of the spectrum, as limited by the ocular shutter, observed at any one time, has an effect upon the absorption measurement. In the green, where the absorption band is comparatively wide, no noticeable differences are encountered up to a width of about 8.0 mμ. In the narrower yellow band, the effect is greater and the values for absorption are correspondingly less reliable. Included in figure 2, are lines drawn to indicate the limits of spectrum used by several investigators. If these be projected on the absorption curve, it is easily seen that wide ocular shutter limits include light from the "edges" of the band which would, therefore, lower the absorption values.

By removing the spectrometer, the adjustment of the photometer could be studied separately. First, it was noted that the dividing line of the biprism could be brought sharply into focus. The ground glass of the lamp house was then removed, also the eyepiece of the photometer, so the image of the lamp filament could be seen through the photometer. By rotating the nicol, it was found that this image could be extinguished at points on the analyzing nicol scale corresponding exactly to 90°, 180°, 270° and 360° or 0°, which proved that the instrument was in proper

adjustment. To guard against the possibility of the adjustment changing while making measurements, the specimens were occasionally analysed in duplicate using opposite quadrants of the photometer. No discrepancies were encountered.

Method of obtaining optical density (D). The general procedure adopted in making the spectrophotometric absorption measurements was as follows: Blood previously analysed for hemoglobin content was diluted accurately by a known amount (usually 1–100 in 0.4 per cent ammonia), and a cell 1 cm. thick was filled with the solution. A similar cell was filled with the solvent. (Part of the blood solution was saturated with illuminating gas and reserved for further spectrophotometric examination.) The spectrophotometer was adjusted properly and the cells containing the specimen and solvent were placed in the two light beams. The wavelength was set at the desired point and the analysing nicol of the photometer was

TABLE 4

OCULAR SHUTTER SCALE	SHUTTER WIDTH IN mμ	DENSITY OBSERVED
Green band—540 mμ (mean)		
0.5	3.5	1.04
1.0	5.5	1.04
2.0	8.5	1.02
Yellow band—575 mμ (mean)		
0.5	4.5	1.11
1.0	7.0	1.10
2.0	10.0	1.06

rotated until a match in the comparison field was secured. The total intensity in the observation field was adjusted by the collimator slit to give a dim field, but one easily read. Record was made of the degrees on the analysing nicol scale, of an average of five repeated settings. The wavelength was changed and similar readings taken until the range of the visible spectrum was covered. The cells containing the solution and solvent were then reversed and the entire spectrum was read as before. The individual readings did not vary more than 0.2 to 0.3° at any one wavelength except at the extremes of the spectrum.

The usual formula given for a Martens type of photometer is

$$T = \frac{I'}{I} = \tan^2 \theta \qquad (1)$$

where T is the transmission; I, the intensity of incident light; I', the intensity after passing through a solution, and θ the angle of rotation of

the analysing nicol. The above formula assumes that the zero point is exactly 45°, and also assumes that the substances under test were optically inactive. It is one of the principal advantages of the Martens type of photometer that the effects of any polarization due to the specimen and of any deviation of the match point from 45°, can be completely compensated by taking two sets of readings with the specimen, first in one field and then in the other. If θ_1 is the scale reading of the analysing nicol for match when the sample is covering the half of the field which is extinguished for $\theta = 0°$, θ_1 will vary from 45° to 90°. If θ_2 is the scale

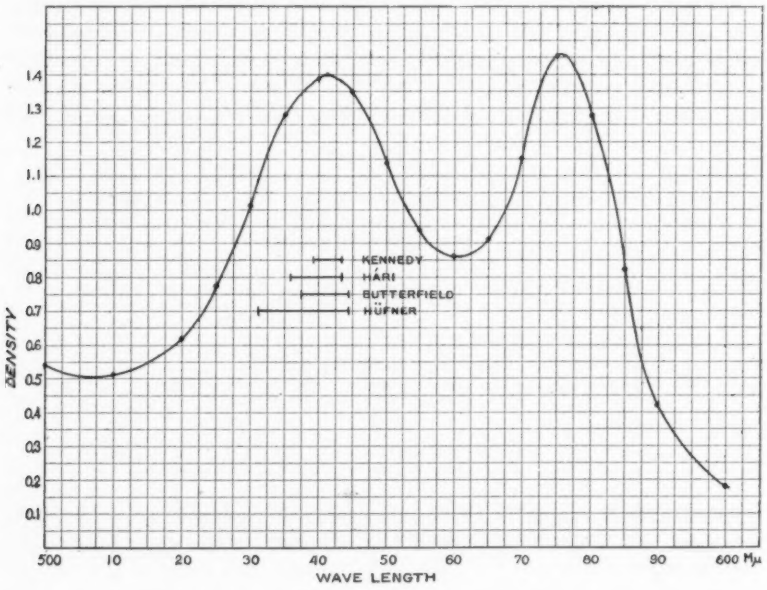

Fig. 2

reading of the nicol when the sample is covering the half of the field which is extinguished by $\theta = 90°$, θ_2 will vary from 0° to 45°.

$$T = \frac{\tan \theta_2}{\tan \theta_1} \tag{2}$$

$$= \tan \theta_2 \cdot \cot \theta_1 \tag{3}$$

In measuring the amount of light absorption in solutions, reference is almost always made to thickness and concentration. It seems more logical, therefore, to express absorption in terms that are directly pro-

TABLE 5

Absorption at different wavelengths in the yellow band (α)

MEAN WAVE LENGTH	ABSORPTION IN TERMS OF OPTICAL DENSITY				
	Dog blood		Human blood		Donkey blood
	c = 2.13	c = 1.62	c = 2.11	c = 1.62	c = 1.13
mμ					
573	1.76	1.40	1.77	1.38	0.94
574	1.85	1.44	1.82	1.43	0.97
575	1.89	1.46	1.87	1.45	1.00
576	1.91	1.44	1.89	1.45	1.00
577	1.90	1.39	1.88	1.41	1.00

Ocular shutter = 3.0 mμ.
(1.0 cm. cell)
Maximum absorption occurs between 575 and 577 mμ.
Hári, 575.6 s.p.
Heubner and Rosenberg, 576.9 s.p.
Formanek, 578.1 p.
Schumm, 578.0 p.

Absorption at different wavelengths in the green band (β)

MEAN WAVE LENGTH	ABSORPTION IN TERMS OF OPTICAL DENSITY				
	Dog blood		Human blood		Donkey blood
	c = 2.13	c = 1.62	c = 2.11	c = 1.62	c = 1.13
mμ					
				1.34	0.91
537	1.74	1.32	1.73	1.36	0.93
538	1.78	1.38	1.76	1.38	0.95
539	1.83	1.39	1.79	1.39	0.96
540	1.84	1.39	1.81	1.39	0.97
541	1.83	1.38	1.80	1.37	0.96
542	1.84	1.37	1.77	1.36	0.96

Ocular shutter = 2.2 mμ.
(1.0 cm. cell).
Maximum absorption occurs between 539 and 542 mμ.
Hári, 540.4 s.p.
Heubner and Rosenberg, 540.2 s.p.
Formanek, 541.7 p.
Schumm, 542.4 p.

TABLE 5—*Concluded*

Absorption between (α) and (β) bands

MEAN WAVE LENGTH	ABSORPTION IN TERMS OF OPTICAL DENSITY				
	Dog blood		Human blood		Donkey blood
	c = 2.13	c = 1.62	c = 2.11	c = 1.62	c = 1.13
mμ					
557	1.16	0.88	1.12		0.63
558	1.14	0.86	1.11	0.89	0.62
559	1.13	0.85	1.09	0.86	0.62
560	1.12	0.85	1.08	0.86	0.60
561	1.14	0.86	1.10	0.85	0.62
562	1.14	0.88	1.11	0.87	0.62
563	1.17	0.89	1.13	0.87	0.62

Ocular shutter = 2.7 mμ.
(1.0 cm. cell).
Minimum occurs between 559 and 561 mμ.
c = concentration in mgm./cc.
s.p. = spectrophotometric.
p. = photographic.

portional to concentrations and thickness, rather than as percentage transmission. For this reason all absorption measurements here are expressed in terms of optical density (D), which may be defined as the negative log of transmitted light, or

$$D = -\log T$$

$$= \log \tan \theta_2 + \log \cot \theta_1 \qquad (4)$$

If concentration c, and thickness t, be taken into account, then a specific density may be expressed as

$$D_s = -\log_{10} \sqrt{c.t}/T \qquad (5)$$

The determination of the absorption maxima of blood solutions. There are three important points in any absorption curve for oxyhemoglobin; that corresponding to the maximum in the green, the maximum in the yellow and the minimum between these maxima. These may be taken as reference points at which the optical constants may be determined with greatest accuracy. Also, the ratio of the maximum absorption in the green to the minimum in the green yellow, which is constant, has frequently been used to identify a pigment as oxyhemoglobin. It becomes important, then, to ascertain what wavelengths are absorbed most and also what wavelengths are absorbed least between these maxima. Various samples of blood were diluted 1:100 with 0.4 per cent ammonia and examined

spectrophotometrically over the regions of maximum and minimum absorption. The results are tabulated, together with some values of other observers.

The maximum (or minimum) density values which are grouped, in table 5, constitute figures which may be considered identical because of the limits of the method. Different samples of blood were used because of the recent work of J. Barcroft (1924) which tends to reopen the question of color differences in the hemoglobin of different species. In this work, no differences have been encountered to support Barcroft's findings.

The ratio of the maximum in the green to the minimum in the green yellow has been frequently used to identify the purity (as far as color is concerned) of hemoglobin solutions. From figure 2, the ratio of the densities at 540 mμ and 560 mμ is:

$$\frac{D_{540}}{D_{560}} = \frac{1.40}{0.86} = 1.63$$

In the very excellent paper by P. Hári (1917) a value "somewhat over 1.60" was obtained. This was higher than the values obtained by previous workers. Hári accounts for the discrepancy by showing the effect of time in methemoglobin formation with correspondingly lower ratio values. The still higher value obtained in this work is probably due to measurements being made with narrower spectral fields.

The determination of the absorption ratio (*A*). Nine different samples of blood were analysed for hemoglobin content by the oxygen capacity method of Van Slyke. The blood was diluted 1:100 with 0.4 per cent ammonia in calibrated glassware and a cell 1 cm. thick was filled with the blood solution and another cell was filled with the solvent. Density measurements were made over the entire visual spectrum, changing the wavelength by steps of 5 mμ. The ocular shutter remained fixed at 3.0 mμ at the Hg green line. The average of five settings of the analysing nicol were taken and density calculated by the method already given. From the known concentration and density determined optically, the absorption ratio was calculated for each wavelength by:

$$C = A \cdot D$$

The values of (A) for a particular wavelength were taken from all the samples analysed and were averaged and the per cent deviation from the mean calculated. The results are summarized in table 6.

The curve in figure 2 is constructed from the values of (A) above together with those derived from the experiments on the points of maximum and minimum absorption. By dividing any value for concentration (in this case 1.635 mgm. per cm.^{3}) by these values for A, the value of density

is obtained and points on the curve plotted. As a result the curve in figure 2 represents an ideal absorption curve for oxyhemoglobin plotted with wavelength as abscissae and degree of absorption as ordinates.

Factors affecting the value of the absorption ratio (A). The type of instrument used for the absorption measurement has been suggested as a possible factor which would account for the apparent disagreement in the optical constants for hemoglobin, (Butterfield, 1919; Gallerani, 1920). P. Hári (1919) has investigated the question and concludes that absorption

TABLE 6

Average value of A at various wavelengths for blood solutions

WAVE LENGTH	OXY-HEMOGLOBIN (A) $\times 10^{-3}$	CO-HEMOGLOBIN (A) $\times 10^{-3}$	ERROR
$m\mu$			*per cent*
460	1.42	1.91	3.5
470	1.85	2.36	1.6
480	2.31	2.69	1.3
490	2.71	2.92	3.0
500	3.01	2.99	2.7
510	3.13	2.78	3.5
520	2.63	2.05	3.8
525	2.09	1.66	2.4
530	1.60	1.36	1.9
535	1.30	1.20	3.1
540	1.165	1.15	1.7
545	1.21	1.22	1.7
550	1.43	1.38	2.1
555	1.74	1.46	1.7
560	1.90	1.42	2.1
565	1.79	1.27	1.7
570	1.42	1.16	2.8
575	1.15	1.31	3.4
580	1.28	1.85	4.7
585	1.88	2.91	7.5
590	3.72	5.26	8.1
600	10.12	13.50	20.0

values are independent of the instrument. However, the observations were made on two photometers, both of the Martens type, so that the question of optically active solutions was not taken into account. In the Martens photometer both light beams are polarized which enables one to make measurements with complete compensation on optically active solutions. It is, however, quite probable that the optical activity occurring within physiological limits, is of no material importance, even if other types of polarization photometers are used. A general comparison and the relative accuracy of various instruments is found in the Bureau of Standards Technical Papers 1919, 1920.

The extent of the spectrum in the observation field of the instrument is probably the most important factor in absorption measurements, especially if the bands are narrow. The effect on absorption measurements of different ocular shutter widths has been shown above. The apparatus used in this work was so admirably constructed for using narrow fields, it was physically impossible to use such wide areas as recorded in older work and so a direct comparison has, therefore, been impossible.

It is a well-known fact that a change in temperature alters the character of absorption spectra. In this work, it was impossible to control the temperature, but record was kept of the solutions when they were examined and a variation of 7.0°C. over the whole series is noted. A temperature change during the examination of any one specimen was never more than 1.5°C.

The question of the effect of solvent for hemoglobin has been discussed elsewhere and it has been shown that the color is the same when water or a weak alkali is used as the solvent (Hári, 1919). Also, Butterfield (1912) has shown that solutions of hemoglobin obey Beer's law, 1852.

Finally, the method of analysis adopted in the determination of hemoglobin concentration is an obvious factor. In this work, the Van Slyke method of analysis was chosen, and the results are correlated with other work where the analysis depended on weights of crystalline material.

ERRORS. In table 6, are listed the average values obtained for the absorption ratio of blood solutions and the corresponding error in per cent. The figures for error were derived in the usual way by the method of least square and they represent the practical issue of this experiment, including errors from all sources. The variation in the error follows from what might be expected, namely, an increase, at the extremes of the spectrum, at the "edge" of absorption bands and also when density values are low. Beside these practical considerations, there remain certain theoretical limits which apply to any subjective photometric measurement.

It has been found, as a result of many trials and in many ways, that the eye cannot distinguish a smaller difference in intensity than about 2 per cent of the total (Helmholtz, 1924). With solutions, a 2 per cent error in intensity is not immediately significant as an error in the measurement of the concentration. However, the error in concentration or the relative error in density may be calculated from the relationship expressed in equation (4).

$$D = -\log T$$

Differentiating

$$dD = -\log e \cdot \frac{dT}{T} \qquad (6)$$

Dividing by D for the relative error

$$\frac{dD}{D} = \frac{\log e}{\log T} \cdot \frac{dT}{T} \tag{7}$$

$$\text{or per cent error in } D = \frac{0.4343}{\log T} \times \text{per cent error in } T \tag{8}$$

If, from physiological considerations, the per cent error in T is 2.0, then substituting for various values of T found in any measurement, the corresponding errors in density are obtained. (See column 7, table 7.) These values may be considered "unavoidable" errors due to the physiological limits of the eye.

Aside from the limits of a physiological nature, the type of apparatus used for the photometric measurement has a certain limit of accuracy. The photometer used in this work has a scale on the analysing nicol which can be set to 0.1°. The relative error in density corresponding to a change of 0.1° can be calculated from equation (4) above. If the match point is considered exactly 45°, then,

$$D = 2 \log \tan \theta \tag{9}$$

Differentiating for the change in density (D) with respect to θ,

$$\frac{dD}{\theta} = \frac{4 \log e}{\sin 2\theta} \tag{10}$$

and dividing by D for the relative error we have

$$\frac{dD}{D} = \frac{2 \log e}{\sin 2\theta \, (\log \tan \theta)} \cdot d\theta \tag{11}$$

If dθ be 0.1°, then substituting various values of θ found in any measurement series, the corresponding error in D is obtained. (See column 2, table 7.) These values are significant enough and show why readings between $\theta = 60°$ and $\theta = 80°$ are the most reliable—a fact which is gained in practical experience.

Returning to the question of the physiological limits of the eye (which has been set at 2 per cent), it is possible to calculate what angle setting of the analysing nicol on the Martens photometer will give just a 2 per cent difference in intensity. Such values are tabulated in column 5. If these values be substituted for $d\theta$ in (11), the result tabulated in column (6), represents the "physiological error" calculated in a different way. Beyond 50° the angles are increasingly less than 0.1°. Column 6 and 7 represent values for unavoidable error derived in two ways; first, by assuming a physiological error of 2 per cent error in transmission and calculating the

corresponding error in density (which is proportional to concentration) second, by calculating the error resulting from a change of 0.1°, which is the smallest division on the scale of the photometer.

From equation (4), it is found that a 2 per cent difference in transmission is equivalent to a difference of 0.0086 in density. This value is constant and represents the least difference detected by the eye. In figure 3, this value is plotted as per cent of density for various values of density and serves as a convenient reference for "physiological" errors in any subjective photometry. The two lines correspond to an assumption of 1 or 2 per cent error in transmission, as the case may be. In any absorption measurements where there is an interest in concentration, errors expressed in terms

TABLE 7

(1) Θ	(2) ERROR PER CENT FOR $d\Theta = 0.1°$	(3) DENSITY	(4) TRANSMISSION	(5) $\Theta \Rightarrow 2$ PER CENT T	(6) COLUMN 2 × COLUMN 5	(7) CALCULATED FOR 2 per cent T $\Rightarrow 0.0086D$
			per cent		*per cent*	*per cent*
46°	10.24	0.030	93.3	0.29°	29.7	28.6
47	5.00	0.061	87.0	0.26	13.0	14.3
48	3.34	0.091	81.1	0.20	6.7	9.5
50	2.02	0.152	70.4	0.10	2.0	5.7
55	1.04	0.310	49.0	<0.10	*	2.8
60	0.74	0.477	33.3	<0.10	*	1.8
65	0.60	0.663	21.7	<0.10	*	1.3
70	0.54	0.878	13.2	<0.10	*	1.0
75	0.54	1.144	7.2	<0.10	*	0.7
80	0.58	1.507	3.1	<0.10	*	0.6
85	0.82	2.116	0.8	<0.10	*	0.4
87	1.14	2.561	0.3	<0.10	*	0.3
88	1.50	2.914		<0.10	*	0.3
89	2.48	3.516		<0.10	*	0.2

* Same as column 2.

of density are significant while those in transmission have no direct bearing on concentration values.

Discussion. Although a great deal of experimental work has already been done on the determination of certain optical constant for hemoglobin, yet in most cases the analyses for hemoglobin depended on weights of crystalline material. It seemed, therefore, valuable to repeat previous work using another method of hemoglobin analysis, namely, that perfected by Van Slyke and co-workers (1924). Although Newcomer used Van Slyke's method of hemoglobin analysis, his absorption measurements were made with the disadvantage of a quartz spectrograph (1919). Besides the method employed for hemoglobin analysis as a possible reason for the discrepancies encountered in previous work, the spectral regions employed

seem unusually broad. With the modern optical instruments, it is possible to work with much narrower fields and therefore more reliable results are possible. The importance of size of observation field has been emphasized.

A determination of the position of maximum absorption has been described with only slight reference to some very beautiful spectrophotographic work done by Schumm (1913), Rost (1909) and others. Even

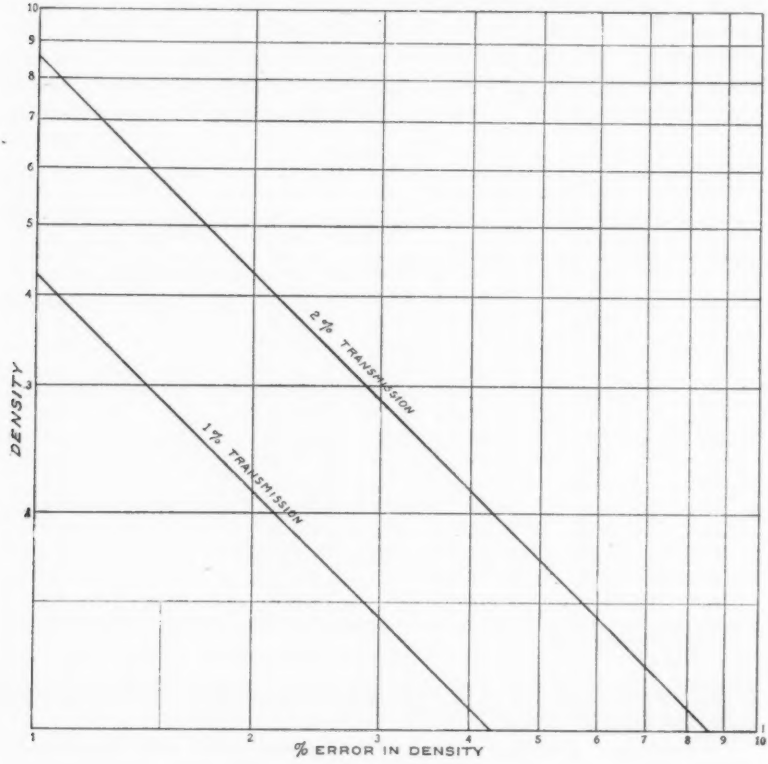

Fig. 3

though the spectrophotometric method would certainly be the one of choice, for the determination of the position of absorption maximum, the results of both methods are in close agreement.

Beside the actual errors encountered in the work, a theoretical basis for certain errors has been included. A consideration of these is not without some practical importance. In a recent publication by Mann (1926) there is an attempt to show the presence of hematin in blood serum from the consideration of points on a spectrophotometric curve where the trans-

mission is about 90 per cent. Under these conditions the error of measurement is upward of 30 per cent and any interpretation, therefore, should be made with some reserve.

SUMMARY

1. The analysis of hemoglobin solutions has been carried out by the oxygen capacity method and the results correlated with the quantitative measurements of light absorption by such solutions.

2. The absorption ratio for hemoglobin has been determined over the visual spectrum and a curve plotted. The values agree with those obtained by some other investigators where the analysis depended on the weight of crystallized oxyhemoglobin.

3. Certain precautions and limits of error in spectrophotometric measurement are discussed.

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STUDIES ON DIABETES INSIPIDUS. I

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The present status of the knowledge of diabetes insipidus leaves three fundamental phases of the problem more or less unsolved, namely, the exact site of the causative disturbance, the nature of that disturbance, and the way in which it produces the diuresis.

The disorder is considered to be due to a pathological condition of the hypophysis by some investigators and to a disturbance of the floor of the third ventricle by others. The evidence in favor of the latter and most recent view is briefly as follows: Camus and Roussy (1913), Roussy (1925), Bailey and Bremer (1921), Hanchett (1922) and Curtis (1924) have produced typical diabetes insipidus in experimental animals by injury to the floor of the third ventricle without injury to the hypophysis but not by injury to the hypophysis alone (Hanchett, 1922) or by hypophysectomy unaccompanied by injury to the floor of the third ventricle (Roussy, 1925). Roussy and Lévy (1925) and Curtis (1924) conclude that the tuber cinereum is the site of the effective lesion. Bailey and Bremer (1921) suggest that the mammillary bodies may also be involved. A very complete bibliography of the extensive literature on this phase of the problem may be obtained from the papers on diabetes insipidus by Allen and Sherrill (1923) and on the pituitary body by Geiling (1923) who discusses the evidence for the views that the hypophysis is responsible for the disorder.

The question whether diabetes insipidus is an irritation or deficiency phenomenon has received little serious attention and no specific investigation. In general those who refer the condition to the hypophysis consider that it is caused by a deficiency in the secretion of the posterior lobe or pars intermedia (Geiling, 1926) though Dott (1923) attributes it to irritation of the pars intermedia. Roussy and Lévy (1925) conclude that it is impossible to say whether the lesion is one of hemorrhage or injury. Bailey and Bremer (1921), on the other hand, think that the evidence is in favor of the hypothesis that diabetes insipidus is a deficiency phenomenon. The facts on record, both clinical and experimental, may be

interpreted in either way though they suggest that it is irritative for experimental diabetes insipidus is usually transitory and can be reinduced at will, clinical diabetes insipidus may be chronic or recurrent, and in chronic cases which have come to autopsy the presence of something which might well be a source of irritation such as a tumor, a clot or a projectile is always reported.

Whatever its nature, there are five obvious ways in which a lesion to the floor of the third ventricle might produce diuresis. 1, It might influence the hypophysis directly; 2, alter kidney function through its extrinsic nerve supply, 3, lead to the ingestion of an excessive quantity of water by causing thirst, or 4, it might change the composition of the blood by way of a peripheral organ or organs after the manner of the puncture of Claude Bernard. Finally, 5, a substance acting directly or indirectly as a diuretic might be produced at the site of lesion as a result of the disturbance caused by the injury.

Though the part played by the hypophysis is still under discussion, there is much evidence that it is neither involved directly nor indirectly. The second possibility has been eliminated for several investigators have found that denervation of the kidney does not stop or prevent the development of the diuresis (Bailey and Bremer, 1921), (Camus and Gournay, 1924), results which have been confirmed incidentally in the course of present investigation.

It is uncertain whether the thirst is primary and therefore responsible for the diuresis, or secondary to it. Bauer and Aschner (1920) have left the question open. The majority of investigators report that the thirst precedes the diuresis. Apparently on the basis of similar observations, and the fact that water restriction produces a nearly proportionate decrease in urine output, Curtis (1924) concludes that the thirst is primary. Roussy (1925) and Bailey and Bremer (1921) agree that at times polydipsia precedes polyuria and that at other times the reverse is true, results which demonstrate that at least in some cases the polyuria is primary. None of these investigators describe the experimental procedure from which they draw their conclusions.

The fourth and fifth possibilities, namely, that the diuresis is due to blood changes produced by way of the somatic or autonomic nervous systems or by a chemical formed at the site of lesion, have not been investigated to the knowledge of the writer. The attempts, which have been made thus far, to explain the diuresis on the basis of blood changes other than those produced by ingestion of water or an upset in hypophyseal function, have been confined to efforts to determine the chemical nature of that change rather than the stimulus immediately responsible for its production.

The present investigation has been concerned with the phases of the problem discussed in this introduction.

METHODS. *Production of diabetes insipidus.* Dogs were used throughout this investigation. The hypophysis was exposed by the method described by Aschner (1912) with minor changes and a small electric cautery made of a loop of platinum wire soldered to a pair of insulated electrodes was slipped dorsally past its posterior border then directed forward to lie between it and the mammillary bodies. The cautery loop was heated sufficiently to produce slight injury rather than destruction.

Record was kept of the water intake and urine output of each animal and frequent tests for glucosuria were made by Benedict's qualitative method. No effort was made to standardize the diet in any of the experiments.

Lesions. If diabetes insipidus is an irritation phenomenon, it should be produced by slight injury to the proper area but not by depression or destruction of that area. If it is a deficiency phenomenon, the reverse should be true. This was tested by comparing the effects of various periods of cauterization of the floor of the third ventricle on the urine output in a series of sixty-eight dogs. The exact site and extent of the lesions were ascertained by careful post-mortem examination of the brain of each animal. For this purpose serial sections of the first twenty-one brain stems and hypophyses were prepared by the usual histological technique. In the remaining experiments, the data was obtained from free-hand sections of the material after it had been hardened in 10 per cent formalin.

Depression of midbrain. Further evidence in the same direction was obtained by depressing the midbrain, and therefore presumably the hypothalamus, of a series of diabetic dogs by means of drugs, those used being morphine sulphate, $\frac{1}{2}$ to 2 mgm. per kilo, ether anesthesia for periods of from 5 to 30 minutes, and sodium barbital, 0.25 gram per kilo. Urine was collected through a retention catheter for 4 or 5 control periods of 20 minutes' duration. The drug was then administered, and the urine collected through a period of two to three hours. Water was withheld during the experiment. Inasmuch as such procedure might stop the diuresis by interfering with the functioning of the kidneys, or with the absorption of water from the intestines, or by depressing an existing irritation in the central nervous system, the results were controlled by intravenous injections of a 10 per cent solution of sodium sulphate and by water deprivation experiments.

Hypophysectomies. Because the part played by the hypophysis in the production of diabetes insipidus is still disputed, a group of puppies between $1\frac{1}{2}$ and 2 months of age were hypophysectomized. This was done by removing as much of the gland as possible with a small pair of curved forceps and then cauterizing the remaining stump. In part of the experiments care was taken to avoid injuring the floor of the third ventricle. In the others it was purposely cauterized. The results of each operation were determined by recording the pre- and post-operative urine output and

by histological examination of the brain stem with the exception of that of no. 9 in which complete hypophysectomy was unmistakable to the naked eye.

Thirst. The diuresis of diabetes insipidus should be dependent on, and directly proportional to, the water intake if the thirst is primary. If the thirst is secondary, it should lead to a more or less rapid desiccation of diabetic animals deprived of water, and should develop spontaneously in operated dogs restricted to their normal water intake. Accordingly one series of dogs, in which diabetes insipidus was already established, were deprived of water for periods of from 12 to 24 hours. A second series were restricted to their average pre-operative water intake for a period of 24 hours following the cauterization of the region of the mammillary bodies or until an unmistakable diuresis had developed. In order to detect any desiccation and to obtain a true estimate of the water lost to the body, the water intake, the volume and depression of freezing point of the urine, the red blood corpuscle count, serum depression of freezing point, and rectal temperature were recorded at intervals before, during, and after the period of water restriction. In 13 experiments the whole blood and serum chlorides were also determined by the Van Slyke (1924) method. Food was withheld during the course of these experiments and for a period of 12 hours preceding each chloride determination. Blood was drawn from the external saphenous vein in all cases.

Somatic and autonomic nervous systems. The possibility that the diuresis is due to blood changes produced by a peripheral organ in response to changes in the somatic or autonomic nervous system was tested by studying the effects of eliminating the various nerve paths on the diuresis of diabetic dogs. This was accomplished by performing high spinal section, by double vagotomy below the diaphragm the success of which was confirmed at autopsy, and by atropinization to the point at which the vagal control of the heart, salivation, and the light reflex disappeared.

Cross transfusion experiments. Cross transfusions were performed from the femoral artery of the diabetic dog to the saphenous vein of a normal dog and vice versa. In all except a few of the early experiments in which the normal dog had received 0.25 gram of sodium barbital per kilo, the vessels were exposed and cannulated under local novocain anesthesia, 2.5 to 7 cc. of a 0.5 per cent solution proving adequate for the purpose. After the cannulae were in place the procedure was similar to that used in studying the effects of depression of the midbrain by drugs. The duration of the cross transfusions was 1 to 1½ minutes as longer periods invariably produced oliguria if not anuria.

RESULTS AND DISCUSSION. *Diabetes insipidus.* The diabetes insipidus produced by cauterization was transitory appearing within 5 to 10 hours after the operation in a majority of experiments though the latent period

TABLE 1

Relation between hypophysectomy, lesions to the floor of the third ventricle and diabetes insipidus. +, lesion present; ++ moderate lesion; +++ extensive destruction

EXPERIMENT	NUMBER OF EXPERIMENTS	DIABETES INSIPIDUS	METHOD OF EXAMINATION OF BRAIN	EXTENT OF INJURY							REMARKS
				Post mammillary	Mammillary bodies	Tuber cinereum	Walls of third ventricle	Hypophysis: Anterior lobe	Hypophysis: Posterior lobe	Hypophysis: Infundibulum	
27		Failed to develop	Histological	++	Destroyed	++	0	No trace	No trace	Present	
28		Failed to develop	Histological	0	+++	++	0	No trace	No trace	No trace	
32		Failed to develop	Histological	0	0	0	0	No trace	No trace	Remnant	Lesion lateral to the mammillary bodies
9		See remarks	Gross	0	0	Largely destroyed	+	No trace	No trace	No trace	This dog was allowed to survive the operation 3 weeks. It was diabetic one day following the operation
	9	Failed to develop	Gross	0 or +	+++	+ to +++	0 to ++	Destroyed	Destroyed	*	*Infundibulum apparently absent
24		Present	Histological	0	+	0	0	Destroyed	Destroyed	No trace	
24		Present	Histological	0	++	0	+	Destroyed	Destroyed	No trace	
25		Present	Histological	0	+	+	0	*	Destroyed	No trace	* Fragments of the anterior lobe in a superficial clot
26		Present	Histological	0	++	+	+	No trace	Destroyed	No trace	
45		Present	Histological	0	+++	+	0	No trace	*	Present injured	* Portions of charred lobe left. No normal tissue detected
	6	Present	Gross	0 or +	+	+ to ++	0 to +	Destroyed	Destroyed		Infundibulum apparently absent

varied from two hours to as long as forty-eight hours in cases of severe injury. In the experiments in which it developed promptly, the diuresis was most intense during the first few hours following its onset after which it gradually subsided over a period of several days in the case of older dogs. In puppies as young as 1 or 2 months, it ran its course in twenty-four to forty-eight hours.

It is of interest in this connection that hyperglycemia probably often accompanies the diabetes insipidus as is shown by the fact that in several experiments in which the urine gave negative tests for glucose as long as the animal was allowed water ad libitum, it became glucose positive after a period of water deprivation had reduced the urine output to a more nearly normal volume.

Hypophysectomies. A deficiency in the secretions of the hypophysis is not responsible for diabetes insipidus as is evidenced by the fact that the condition failed to develop in 4 hypophysectomized puppies in which complete destruction was verified by histological methods in 3 cases and was unmistakable in the fourth, and in 9 adult dogs in which gross inspection showed that the gland was essentially if not completely destroyed (table 1). These results agree with those recorded by Roussy (1925) and elsewhere in reviews of the literature (Bauer and Aschner, 1920), (Veil, 1918).

On the other hand, diabetes insipidus would not seem to depend upon any secretion of the hypophysis for its development in 5 puppies, in which complete hypophysectomy was verified by histological technique, and in 6 dogs, in which gross inspection gave no evidence of its presence (table 1). These results, however, do not preclude the possibility that it is caused by irritation of the tissue in the floor of the third ventricle, which Houssay, and Abel and Geiling (Ceiling, 1926) claim to be capable of secreting the hormone of the posterior lobe. If that is the case, the irritation is indirect as indicated by the experiments reported by Hanchett (1922) who found that irritating injury to the hypophysis alone does not produce diabetes insipidus and by the experiments reported in the next section of this paper.

Lesions and depression of the midbrain. Examination of the brains of 35 animals sacrificed while severely diabetic, that is while passing 80 cc. or more urine per hour in the case of 10 to 15 kilo dogs, uniformly revealed slight injury to one or both mammillary bodies amounting to a localized clot or superficial destruction (table 2, groups 1, 2 and 3). No other lesion was detected in 11 of these experiments. In 5 other experiments in which the diabetes insipidus though present was very mild and slow to develop, the lesion amounted to extensive destruction of the mammillary bodies together with more or less injury to the tuber cinereum (table 2, group 4).

The animals which failed to develop diabetes insipidus following cauteri-

TABLE 2

The relation between the place and extent of lesion and the development of diabetes insipidus. +, lesion present; ++, moderate lesion; +++, extensive destruction

GROUP	NUMBER OF EXPERIMENTS	DIABETES INSIPIDUS	EXTENT OF INJURY					REMARKS
			Post mammillary	Mammillary bodies	Tuber cinereum	Walls of third ventricle	Hypophysis	
1	11	Severe	0	+	0	0	0 or +	80 cc. or more of urine per hour for 11–15 kilo dogs
2	15	Severe	0 or +	+	+	0 or +	0 or +	
3	9	Severe	0 or +	+	0	+	Destroyed or ++	
4	5	Mild	+	++	+++	+	+++	500–700 cc. of urine per 24 hours, weight 9–15 kilos
5	3	Fails to develop	0 or +	0	+	0	0 or +	
6	3	Fails to develop	0	0	0	0	0 or +	Lesions lateral to the mammillary bodies
7	1	See remarks	0	0	Largely destroyed	++	Destroyed	This dog was allowed to survive the operation 3 weeks. It was diabetic for one day following the operation
8	8	Fails to develop	0 or +	Destroyed	++	0 or +	0 to ++	
9	5	Fails to develop	+	+++	+++	+	+++	
10	8	Fails to develop	0 or +	+	0 to +++	+	+++ or destroyed	

zation of the hypothalamus fell into two groups the first of which was comprised of 7 dogs receiving injury to the floor of the third ventricle elsewhere than in the mammillary bodes which were normal in appearance (table 2, groups 5, 6 and 7). No. 9 is of particular interest in that every vestige of the hypophysis and most of the tuber cinereum were destroyed without any material injury to the mammillary bodies, which appeared to be normal at the time of autopsy. This dog suffered a slight diabetes insipidus on the day following the operation, after which the urine output remained normal for the three weeks it was under observation.

The second group is comprised of 21 animals subjected to prolonged periods of cauterization which resulted in asthenia and in practically complete destruction of the mammillary bodies or in severe injury to the entire floor of the third ventricle (table 2, groups 8, 9 and 10). The failure of these animals to develop diabetes insipidus was in no cases due to the moribund condition of the animal. All of group 8 recovered more or less completely from the operation and only those experiments were included in groups 9 and 10 in which the animals were in good condition. An existing diabetes insipidus was stopped in 3 other animals by such treatment, two of which recovered completely from the post-operative asthenia.

In other words, diabetes insipidus follows slight injury to the mammillary bodies but not injuries elsewhere in the floor of the third ventricle or severe injury accompanied by destruction and depression, results which justify the conclusion that the mammillary bodies are the site of effective lesion, and that diabetes insipidus is an irritation rather than a deficiency phenomenon.

The latter conclusion, which has important theoretical and clinical implications, is supported by the results of depressing the midbrain with drugs. Morphine in doses stimulating the medulla, and ether and barbital in doses less than anesthetic, augment the diuresis, 6 experiments. The same drugs in doses just sufficient to depress the midbrain reduce the urine output to normal as soon as the depression sets in, 10 experiments. Suppression of the diuresis is not due to interference with renal secretion for saline diuresis can be produced at any time. It is not entirely due to interference with absorption from the alimentary canal for, though the water intake and urine output of highly diabetic dogs are closely parallel, the per minute volume urine output is still well above normal after 16 to 20 hours of water deprivation. It is well known that anesthesia stops the diuresis of diabetes insipidus but the augmentation produced by smaller doses of these drugs and the significance of these correlations seems to have been ignored or considered unimportant.

Thirst. Diabetes insipidus developed during the period of water restriction in 13 of 19 experiments in which animals were kept on their normal pre-operative water intake following cauterization of the region of the

TABLE 3

Effects of water restriction on diuresis, serum Δ, erythrocyte count, and rectal temperature. Experiments 1 to 8, animals restricted to their average pre-operative water intake following cauterization of the mammillary bodies. Experiments 9 to 22, diabetic animals totally deprived of water after a period of water ad libitum. Absolute loss of water, column 6, was determined by substracting the total urine output for the period of water restriction and the preceding control period from the total water ingested during those periods

EXPERIMENT	WEIGHT OF ANIMAL, KILOS	DIABETES INSIPIDUS IN TERMS OF CUBIC CENTIMETERS URINE PER HOUR, WATER AD LIBITUM	PERIOD OF WATER RESTRICTION				PER CENT OF INGESTED WATER EXCRETED IN URINE			SERUM Δ		PER CENT INCREASE IN ERYTHROCYTES	INCREASE IN RECTAL TEMPERATURE, °C.	REMARKS
			Duration, hours	Total urine output in cubic centimeters minus water ingested during period	Absolute water lost in the urine in cubic centimeters	Urine Δ at end of period	Control period, water ad libitum	Control + water restriction period	Period following water restriction, water ad libitum	Water ad libitum	At end of period of water restriction			
1	15.5	236	13	736	726	0.86	62.6	228	66.4	0.63	0.68	0	2.7	Motor incoördination
2	15.6	200	8.5	673	183	0.78	31.2	122			0.78	16.6	2.0	Motor incoördination
3	8.5	410	8	700	620	0.415		207		0.64	0.665	25.7	0	Motor incoördination
4	14.0	142	13	875	375	0.51	85.0	175	50		0.63			
5	24.5	130	24	810	410	1.235	66.6	165	64.4			6.27	1.5	Room temperature 98°F. Severe hemorrhage during operation
6	20.4		24	805	780		30.5	103.0				20.6	3.5	Animal in coma throughout period
7	7.2	103	9.5	150	70	2.18	86.6	111.6	55.5			25.8	0	First 100 cc. of urine had reducing properties. The later urine non-reducing
8	7.2	103	9.0	350	200 [4]	1.12				0.63	0.66	0	1.3	
9	17.5	98	24.5	997	867		97.5	126.4	12.8			24.7	1.5	
10	8.5	430	10.5	960	960		100	116.9	91.0	0.685	0.645	0	0	
11	6.2	118	24.5	488	945	3.39	118.4	137.8	122.0	0.68	0.675	17.5	1.7	

12	6.2	141	24	359	259	1.27*	90.9	123.5	73.6	0.68	0.69	0	0	* Middle of period
13	11.0	69	23	360	324		95.5	140	53.4			3.8	0.8	
14	13.5	131	22.5	720	510	0.91	91.7	113.7			0.66	30.1	1.0	
15	5.0	52	24	554	160		78.2	170						
16	6.5	76	25	406	76	3.58	80.0	140.5	64.5	0.65	0.633		0	
17	19.0	207	13	1080	70	1.85	73.3	101.8	71.4	0.58	0.545	0	2.5	Room temperature 36.6°C.
18	14.0	360	19.5	863	0		77.3	89.6	67.9				0	
		105	25.5	203	0	1.87	81.2	89.0	49.7	0.65	0.675	36.0	0	N-Normal. Control experiment on 20 after recovery from diabetes insipidus
19	5.0													
		N*	26	55	0	3.19	49.8	54.0			0.625	0	0*	
20	13.0	43	17	160	0		58.4	61.2				2.6	0	
21	13.0	112	7.5	1035	0	0.625	69.1	97.1	35.0			25.5	0	Animal much depressed. Rectal temperature drops 0.8°C. Immediate recovery after drinking water
22	17.0	120	14	204		0.815						17 9	0	

mammillary bodies. The diuresis was so intense in 3 experiments that pronounced loss of motor coördination occurred within 3 to 5 hours after its onset and an increase in rectal temperature, erythrocyte count, or both resulted in the eight experiments in which they were recorded (table 3, experiments 1 to 8). These symptoms of desiccation were promptly relieved by the administration of water. Diabetes insipidus failed to develop in the remaining experiments even after water was allowed ad libitum.

TABLE 4

Whole blood and serum chlorides in normal and diabetic dogs, grams per 100 cc.

DOG NUMBER	SERUM CHLORIDES				WHOLE BLOOD CHLORIDES			
	Water ad libitum		Water deprivation		Water ad libitum		Water deprivation	
	Normal	Diabetes insipidus	Normal	Diabetes insipidus	Normal	Diabetes insipidus	Normal	Diabetes insipidus
1	0.5973	0.5747		0.6077	0.5178	0.5286		0.50356
	0.5784	0.5570			0.5083	0.5057		
12	0.5452	0.5974		0.5784	0.4648	0.5027		0.5121
				0.5888				0.5050
9	0.5925	0.59733		0.6232	0.4820	0.5174		0.5593
		0.5689				0.5057		
15	0.5169		0.5784		0.4412			0.4741
10	0.5784	0.5181	0.5689	0.6065	0.4814	0.4814	0.53117	0.5358
	0.5859	0.6183		0.5547	0.5206	0.5442		0.4563
				0.6361				0.5536
2		0.54616				0.5071		
		0.54621				0.4974		
		0.60774				0.5301		
				0 6505	0.5406	0.5217	0.5604	0 5955
19	0.6172	0.6084	0.6481	0.6154			0.5955	0.5691
			0.6505	0.6521				
16	0.5784		0.5736	0.5840	0.4555			
	0.5405				0.4616		0.47902	0.4909
18	0.6219	0.56628	0.5917	0.6281	0.4862	0.52240	0.5240	
			0.5802	0.5896	0.5027		0.5027	
Average.	0.5775	0.5755	0.5986	0.6104	0.4885	0.5137	0.5321	0.5231

These results seem sufficiently consistent to justify the conclusion that the thirst of diabetes insipidus is secondary to the diuresis, which must therefore be due to a diuretic other than water. The explanation of the discrepancy between this conclusion and that of Curtis (1924) who says that the thirst is primary, and of Roussy (1925) who says that sometimes it is primary, sometimes secondary, is not clear. It is suggested that possibly these investigators did not restrict their animals to the normal preoperative water intake for a period long enough to cover the latent period of

development of experimental diabetes insipidus, which varied from 2 to 48 hours in this series of experiments.

The withdrawal of water after diabetes insipidus had become established resulted in desiccation of the animal in 13 of 14 experiments as indicated in 9 by an increase in rectal temperature or erythrocyte count, both of which returned to normal after the ingestion of water (table 3, experiments 9 to 22). In 9 experiments the volume of urine excreted during the period of water deprivation and the 18 or 20 hours preceding it materially exceeded the amount of water consumed during the 18 or 20 hours preceding it.

TABLE 5

Cross transfusion between a normal dog weighing 9½ kgm. and a diabetic dog weighing 11½ kgm. and secreting 124 cc. of urine per hour

TIME	PROCEDURE		URINE OF NORMAL DOG, CUBIC CENTIMETERS PER HOUR
10:25		Urine collection begun	
:40		Urine 1	4.8
:55		Urine 2	4.8
11:05		Urine 3	5.27
:25		Urine 4	5.0
:45		Urine 5	4.2
:55	Cross transfusion begun	Urine 6	4.12
:56½	Cross transfusion stopped		
:58		Urine 7	15.0
12:00		Urine 8	16.5
12:05		Urine 9	0.6
:20		Urine 10	0.54
:40		Urine 11	1.8
1:10		Urine 12	2.4
1:30		Urine 13	3.01
1:45		Urine 14	2.8

In a few experiments the serum Δ also rose but the blood and serum chloride values fell within normal limits (table 4).

These results confirm those obtained by Bauer and Aschner (1920) in a case of clinical diabetes insipidus and those obtained in the first group of water deprivation experiments of this investigation. They differ from those of Curtis (1924) who reports hyperchloremia in diabetic dogs; from those of Bailey and Bremer (1921) who found no evidence of blood concentration after depriving diabetic dogs of water; and from those of Christie and Stewart (1917) who do not find a change in the electrical conductivity of the serum of diabetic patients after such treatment. Several factors may be responsible for these discrepancies, the most obvious being the severity of the diabetes insipidus, the duration of the period of water

deprivation, and the way in which the tissues respond to desiccation in different animals. The importance of the second factor was demonstrated in this series of experiments by the fact that blood concentration, as indicated by increased erythrocyte count, and a change in rectal temperature, did not occur during the first 14 to 20 hours of water deprivation in the second series of animals. The importance of the third factor is illustrated by comparing the results of experiments 1, 2 and 3, table 3. All three of these animals suffered an absolute loss of several hundered cubic centimeters of water in the urine and a pronounced loss of motor coördination but there was no change of erythrocyte count in number 1 or of rectal temperature in number 3.

Somatic and autonomic nervous systems. Diabetes insipidus was elicited in 2 dogs allowed to recover partially from the effects of section of the spinal cord at the level of the second and third thoracic vertebrae. It ran its typical course in 4 diabetic dogs after transection of the cord between the second and fourth thoracic vertebra, and in a fifth dog after transection of the cord at the level of the eight cervical vertebra, in 2 dogs after double vagotomy below the diaphragm, and in 4 dogs after the administration of atropin till vagal control of the heart, salivation and the light reflex had disappeared.

Clearly neither thirst nor the autonomic and somatic nervous systems are responsible for the diuresis of diabetes insipidus. The evidence that a deficiency in the secretions of the hypophysis is not concerned and that the gland proper is not necessary to it is decisive. This would seem to leave only one possibility, namely, that a compound produced at the site of lesion as a result of irritation caused by slight injury acts directly or indirectly as a diuretic.

Cross transfusion experiments. Further evidence of the presence and nature of the diuretic substance is given by the cross transfusion experiments. Cross transfusions between normal dogs for a period of one to one and a half minutes produced no immediate change in the per minute volume output of urine in four experiments and a temporary oliguria in two experiments. Longer periods of transfusion produce oliguria or anuria followed by a prolonged low grade diuresis. This same diuresis followed brief periods of transfusion repeated at one- to two-hour intervals.

Transfusion of blood between diabetic and normal dogs for 1 to 1½ minutes, produced a brief diuresis immediately following the transfusion which lasted 4 to 6 minutes in five experiments, had no effect in two experiments and produced oliguria in two (table 5). The effects of longer periods of transfusion and of repeated transfusions were similar to those obtained on normal dogs. Roussy (1925), on the contrary, reported consistently negative results. The technique was not described.

SUMMARY

1. Diabetes insipidus has been produced in dogs by the cauterization of the floor of the third ventricle.

2. The mammillary bodies are the site of effective lesion.

3. Diabetes insipidus is an irritation rather than a deficiency phenomenon for it is produced by slight injury to the proper area but not by severe injury to or depression of that area.

4. The diuresis must be due to a substance produced at the site of causative disturbance as a result of the irritation, which acts directly or indirectly as a diuretic, for:

a. The hypophysis would not seem to be responsible for it since hypophysectomy without injury to the proper area in the floor of the third ventricle fails to produce diabetes insipidus which, however, is produced in hypophysectomized animals by injury to that area, results confirming Roussy (1925).

b. The thirst of diabetes insipidus is secondary to the diuresis for the diuresis develops in operated animals restricted to their average pre-operative water intake and quickly produces manifestations of desiccation in diabetic animals deprived of water.

c. Neither the autonomic nor somatic nervous systems are concerned in the diuresis for diabetes insipidus runs its typical course after transection of the spinal cord at the level of the eighth cervical vertebra, double vagotomy below the diaphragm, and paralysis of the parasympathetic nervous system with atropin.

5. Cross transfusions between severely diabetic and normal animals performed under novocain anesthesia failed to alter the per minute volume urine output in some experiments, caused a brief diuresis over a period of from 4 to 6 minutes in others. The latter result was never obtained in cross transfusion between normal animals.

I wish to express sincere thanks to Dr. A. L. Tatum, in whose laboratory this work was begun, for suggestions invaluable in overcoming certain technical difficulties and for calling my attention to the importance of the irritative nature of the disorder, to Dr. A. J. Carlson for his generous interest and suggestions, and to Dr. F. C. Koch for the use of his laboratory. I also wish to express most grateful appreciation of the late Dean C. P. Lommen not only for making it possible to pursue this work but for his ever-ready interest and kindly sympathy.

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PITUITRIN AND DIURESIS IN MAN

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Since first noted by Magnus and Schäfer (1901) the influences of pituitrin upon mammalian kidneys have been investigated extensively. Most of these influences have to do with the excretion of water. In the light of this fact it seemed that pituitrin might furnish a precise tool for differentiating among the various stimuli that increase the rate of water elimination, and the results of experiments designed to test this possibility are now to be described.

Schäfer and Herring (1908) showed that intravenous injections of pituitrin caused increases in the rate of output of water by the kidneys. As a result of their experiments it was generally accepted that pituitrin is a "diuretic." But Von der Velden (1913) and Farini and Ceccaroni (1913) were surprised to find that pituitrin evoked no increase in the rate of elimination of water in normal men, and even inhibited excessive eliminations of it such as occur in patients with diabetes insipidus. Where diuresis resulted, all the experiments[1] had been done upon anesthetised mammals, and it has been found that the rate of water excretion augments only in anesthetised or decerebrate individuals.[2]

It was first observed by Von der Velden (1913) that an influence of pituitrin could be demonstrated in a perfectly normal individual if a large quantity of water were ingested. Ordinarily this water is excreted within a very brief time, almost quantitatively (Adolph, 1921), and almost without influence upon the rate of excretion of solutes (Marshall, 1920, 1926). Many experiments in which pituitrin was injected while water was ingested have been reported.[3] In these it was universally

[1] References to these experiments will be found in the publications of Motzfeldt (1917), Rees (1918), Smith and McClosky (1924) and Cushny (1926).

[2] This point has been emphasized especially by Priestley (1921), Richards and Plant (1922), Weir, Larson and Rowntree (1922), Molitor and Pick (1924), Mackersie (1924), Craig (1925) and Cushny (1926). But Molitor and Pick (1925) showed that anesthesia by a proper combination of paraldehyde and chloretone left the effect of pituitrin on water diuresis unaltered.

[3] See the papers of Rees (1918), Houssay, Galan and Negrete (1921), Priestley (1921), Weir, Larson and Rowntree (1922), Molitor and Pick (1924), and Craig (1925).

found that the diuresis was almost completely inhibited for several hours, after which the excessive water was eliminated.

Two experiments of this sort, one with and one without pituitrin, are represented in figure 1. When taken with pure water, pituitrin delayed the excretion of the water for two to five hours. We found in all of the three experiments with pituitrin which we carried out (subjects E, S, L), that at the end of this time the diuresis which came on was not so intense as under control conditions, and the water was not eliminated quantitatively. It is well known (Priestley, 1921; Underhill and Pack, 1923; Miura, 1925) that during this inhibition of diuresis the blood is diluted, and it has been demonstrated on anesthetized mammals (Rees, 1920) that pituitrin does not prevent the absorption of water, though it may

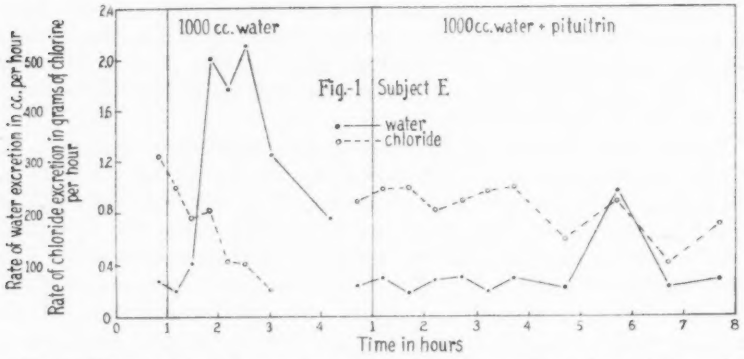
1000 cc. water
1000cc. water + pituitrin
Fig.-1 Subject E.
water
chloride
Rate of water excretion in cc. per hour
Rate of chloride excretion in grams of chlorine per hour
Time in hours

Fig. 1. Effect of pituitrin upon water and chloride excretion after the ingestion of 1 liter of tap water at each of the times marked *1*. In this and the succeeding charts the ordinate values are marked on the time scale in the *middle* of each period of urine collection.

slightly diminish the rate of absorption. There remains some doubt as to whether the effect of the pituitrin in water plethora is directly upon the kidney tissue or upon the circulatory factors in excretion or upon both.[4]

There is evidently need of decisive analysis of the effect of pituitrin upon diuresis in the normal human subject. Certain experiments on man led to the conclusion (Adolph, 1923) that the acceleration of water excretion due to salt ingestion was entirely different in cause and means from that due to water ingestion. In the present experiments we proceeded to make use of pituitrin as an agent for separating the two types of ac-

[4] The evidence for circulatory factors is contained chiefly in the experiments of Schafer and Herring (1908), King and Stoland (1913), Knowlton and Silverman (1918), Cushny and Lambie (1921), Richards and Plant (1922), and Molitor and Pick (1924).

celeration still more sharply. As a result, we are able to state definitely that pituitrin decreases the sensitiveness of the kidneys to the stimuli that usually give water diuresis, but does not inhibit the passage of water through them. The experiments confirm our previous conclusion that the elimination of water in salt diuresis is due not to an excessive concentration of water in the blood, but to the need of water for solute excretion.

EXCRETION OF ISOTONIC SOLUTIONS. The term diuresis usually indicates a temporarily increased rate of *water* excretion. Ordinarily three types are to be distinguished among diureses due to chemical influences: water diuresis, due to the dilution of all solutes in the body; salt diuresis, due to an excessive concentration of some crystalloid in the body; drug diuresis, often due to specific chemical influences upon the renal epithelia. We are concerned in these experiments with an interplay of these three types. Water, salts and drugs may all affect the renal blood vessels, but such influences are undoubtedly less specific than their effects upon the excretory tissues themselves.

Crucial evidence upon the nature of water and salt diuresis was obtained formerly (Adolph, 1923) by ingesting solutions which were approximately isotonic with human blood plasma. It is now known that these isotonic solutions fall into three groups: *1.* Solutions whose solutes are partially or wholly unabsorbed. To this group belong 1.5 per cent calcium chloride, 1.8 per cent sodium sulfate, and presumably isosmotic solutions of all other salts which are not univalent. *2.* Solutions which are completely absorbed but which are slowly or not at all excreted. These are 1.0 per cent sodium chloride, 1.7 per cent sodium bromide, 1.45 per cent sodium bicarbonate, Locke's solution such as that recommended by Douglas and Priestley (1924), and probably solutions of amino acids and carbohydrates such as are absorbed after meals. *3.* Solutions whose solutes and water are completely absorbed and immediately excreted. These are 1.3 per cent potassium chloride, 1.9 per cent urea, 1.45 per cent sodium nitrate, and undoubtedly most other uniunivalent salts and most non-metabolized organic compounds. Their excretion appears to represent simultaneous water diuresis and salt diuresis.

Starting from the results represented in this summary, we performed pairs of experiments upon nine different human subjects.[5] These pairs of experiments, each pair consisting of one with pituitrin and another without pituitrin, were usually performed on adjacent days. Chloride in urine was measured by the Volhard method as modified by Harvey (1910) and urea by the urease method as used by Van Slyke and Cullen

[5] We are obliged to the following for allowing us to make use of experiments which they performed upon themselves: E. L. DuBois, V. Du Vigneaud, J. D. Goldstein, R. F. Lewis, E. J. Manwell, D. O. Meeker and D. J. Stephens.

(1914). The subjects were encouraged to drink sufficient water during the evening before each experiment to ensure complete hydration of the body.

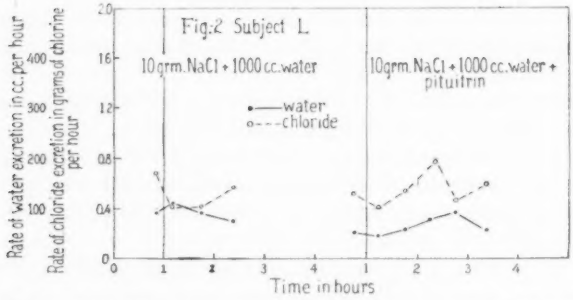
Fig. 2 Subject L.
10 grm. NaCl + 1000 cc. water
10 grm. NaCl + 1000 cc. water + pituitrin
water
chloride
Rate of water excretion in cc. per hour
Rate of chloride excretion in grams of chlorine per hour
Time in hours

Fig. 2. Effect of pituitrin upon water and chloride excretion after the ingestion of 1 liter of 1.0 per cent NaCl at each of the times marked *1*.

TABLE 1

Effects of pituitrin on excretion after ingesting solutions of urea or of KCl isotonic with blood plasma. The rates of solute excretion are expressed in grams of urea and of chlorine respectively

SUBJECT	INGESTA	PITUITRIN	MAXIMAL RATE OF WATER EXCRETION	MAXIMAL RATE OF SOLUTE EXCRETION	TIME, AFTER INGESTION, OF MAXIMAL RATES
			cc. per hour	*gram per hour*	*hours*
K	1000 cc. of 1.9 per cent urea	−	276	−	1.0
		+	102	−	0.5
E	1000 cc. of 1.9 per cent urea	−	673	2.9	1.4
		+	84	1.7	1.5
D	1000 cc. of 1.3 per cent KCl	−	590	2.3	1.4
		+	256	2.0	1.7
M	1000 cc. of 1.3 per cent KCl	−	460	2.5	1.6
		+	210	2.1	1.9
V	600 cc. of 1.4 per cent KCl	−	286	1.7	0.9
		+	154	1.2	1.4

Isotonic solutions of the first group (sodium sulfate) made practically no difference to the kidneys in the excretion of water or of solute as compared to the normal rates; and pituitrin had no measurable effect.

Isotonic solutions of the second group (sodium chloride, fig. 2) showed no effect of pituitrin. The solutions called forth slight and lasting in-

creases in the rate of water excretion, but pituitrin caused no significant modification in these.

Isotonic solutions of the third group were represented by 1.9 per cent urea and by 1.3 per cent potassium chloride. The results of these experiments are shown in table 1, and in figures 3 and 4 (experiments C, D; I, J).

The influence of pituitrin, which was injected intramuscularly at the time of ingestion of one liter of fluid (0.5 cc. of "pituitrin surgical," Parke, Davis & Company), here was extremely marked. Without it the rate of water excretion quickly rose, from a normal of 30 to 60 cc. an hour to a peak as high as 675 cc. an hour. The height and rapidity of rise depended largely upon the emptiness of the alimentary tract. When pituitrin was taken, the rate of water excretion never rose higher than 256 cc. an hour with KCl, and with urea it never rose higher than 102 cc. an hour. The limitation of water excretion was accompanied by a rather slight decrease in the rate of excretion of the diuretic solute in each experiment with pituitrin. The variations among individuals turned out to be surprisingly small.

When pure water was drunk (fig. 1), pituitrin kept the rates of water excretion at or below the rates for control periods. In the case of isotonic solutions which are ordinarily excreted rapidly, this was not true. It is evident that what the pituitrin did was to knock off the part of the diuresis which was due to the excessive water in the body, and to excrete the excessive solute in a relatively concentrated solution. A similar conclusion is furnished by data on isotonic solutions of Oehme (1918), Craig (1925) and Macfarlane (1926). In other words, pituitrin separated the salt diuresis from the water diuresis which otherwise accompanied the excretion of these substances.

Excretion of hypertonic solutions. To test further the conclusion that pituitrin inhibits water diuresis but not salt diuresis, we studied the influence of pituitrin upon the latter. Solutions of urea and of KCl were ingested which were ten times the concentration used in the "isotonic" experiments; and these experiments also were done in pairs, one with and one without pituitrin.

The results of these experiments are given in figures 3 and 4 (experiments A, B; E, F). In no case did pituitrin inhibit the increase in rate of water excretion or of solute excretion which ordinarily followed such ingestion. This fact was previously demonstrated by Motzfeldt (1917), Oehme (1918), Molitor and Pick (1923) and others.

In the two pairs of experiments (A, B; E, F) shown, the maximal rates of water excretion differed less with and without pituitrin than did repeated experiments without pituitrin. In a third pair (subject G) there was an initial rise of water excretion to 244 cc. an hour, which lasted for

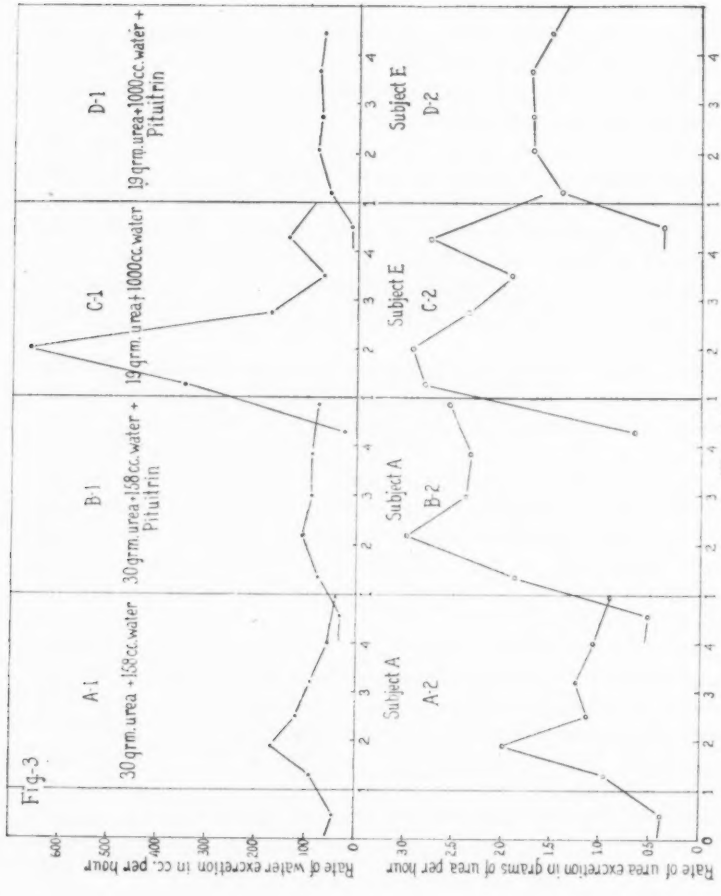

Fig. 3. Effect of pituitrin upon water and urea excretion after the ingestion of $\frac{1}{6}$ liter of 19 per cent urea or of 1 liter of 1.9 per cent urea. Each ingestion and injection was taken at the time marked *1*.

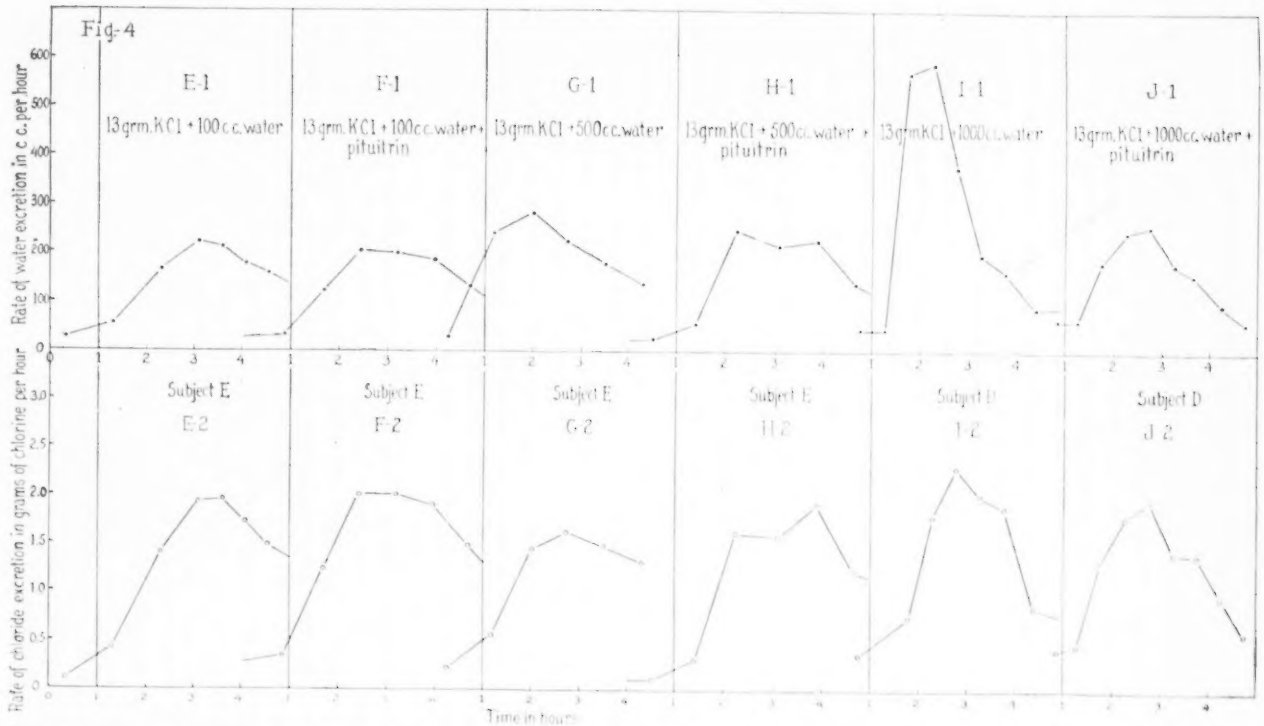

Fig. 4. Effect of pituitrin upon water and chloride excretion after the ingestion of 13 per cent, 2.6 per cent or 1.3 per cent solution of KCl. Each ingestion and injection was taken at the time marked *I*.

thirty minutes, in the experiment where pituitrin was not taken. This initial rise often occurred in salt diuresis experiments (Adolph, 1923), and undoubtedly has to do with temporary redistributions of water during the absorption of strong solutions. This initial excretion of very dilute urine was inhibited by pituitrin. A simulation of such a temporary initial event occurred in an experiment where the pituitrin was not injected until ten minutes after the ingestion had been made.

In salt diuresis the urine was characteristically not concentrated to its maximum with respect to the solute producing the diuresis, until after the peak of water excretion was past (Adolph, 1923). This sequence of concentrations was not affected by the pituitrin, with the exception of the two phenomena just mentioned. The times required for initiating and completing the excessive water excretion were likewise not affected by pituitrin.

The differentiation of salt diuresis from water diuresis may be brought out most clearly by comparing experiments in which identical amounts of solute were taken with varying volumes of water. Two series of such experiments, and the influence of pituitrin upon them, are represented in figures 3 and 4.

The urea series (fig. 3) shows similar curves for water excretion in the two instances when pituitrin was taken, though in one case only 158 cc. of water were ingested while in the other case 1000 cc. of water were ingested. The second pituitrin experiment (D) exhibited a slightly lower rate, but it should be noted that the initial control periods were at unusually low rates in this experiment, and the amount of urea to be eliminated was less. It is clear that with pituitrin the presence of the 1000 cc. of water was completely ignored, and so experiments A, B and D gave similar water excretions.

The potassium chloride series (fig. 4) gives a more thorough comparison. Throughout the three pairs of experiments the amount of salt ingested was constant. With only one-tenth of the water required to make the solution isotonic with blood plasma, pituitrin made not the least perceptible difference. The two experiments G and H, in which one-half of the water required for isotonicity was taken, showed no significant difference between them. As compared with the first pair, the amount of water was increased by 30 and 20 per cent, but this also was within the limits in which experiments duplicate each other. That the water increase was not due directly to the increased water intake is shown by the fact that in each experiment of the second pair, the total excessive water excreted was much above the 500 cc. ingested; obviously there was no water diuresis for pituitrin to inhibit. But in the last pair (isotonic KCl) there was, and with pituitrin the diuresis exactly duplicated that due to a concen-

trated KCl solution. This pair, then, merely illustrates the fact that pituitrin inhibits water diuresis and does not interfere with salt diuresis.

It appears to us that this fact presents great difficulties to the hypothesis of Cushny (1926) that the effects of pituitrin upon the kidneys may be entirely circulatory.

It follows that pituitrin should have, in general, no influence upon the normal renal elimination of water, for it has been shown (Adolph, 1923) that ordinarily in everyday life water is excreted only as a vehicle for solutes.

EXCRETION OF WATER DURING SALT DIURESIS. The relation of the excretion of water to the excretion of substances dissolved in it has been very ill-defined. Yet the influence of water is a potent one in determining the amount of retention (MacKay, 1923) and the time courses of excretion (Adolph, 1921, 1923) of urea, chloride, and probably many other substances. Nevertheless, in general, the upshot of all experiments designed to determine how much the excretion of one substance influences that of others, is that each substance is excreted by the kidneys almost independently of every other. The chief exceptions are represented by the two facts that cations are not extensively excreted without anions, and that no solutes are excreted without water. Both these relationships are obvious necessities, yet difficult problems from the point of view of the organismal economy.

The problem of salt diuresis, then, is one of discovering whether water is excreted because the blood plasma has it in excess (Cushny, 1926) or whether water is excreted because it is required for the convection of solutes. The latter view seems more plausible on account of the fact that a maximal concentration exists for each solute in urine (Adolph, 1923), though it is conceivable that both factors may be present, and that the first may be the means of attaining the second end. Since we do not know with respect to what substance or substances blood dilution is to be measured, it is practically impossible to assert when there exists in the blood an excess of water which will excite the kidneys to a specific excretion of it (Priestley, 1921; Miura, 1925).

Only indirect evidences therefore can tell us whether during a given diuresis the blood contains too much water. One such is the influence of thirst, and it was shown (Adolph, 1921) that thirst does not prevent the production of salt diuresis in man.

A second evidence is furnished by pituitrin. If water is excreted after ingesting a concentrated solution because an excess of water in the blood stimulates the kidneys to its excretion, then pituitrin should abolish the excretion. But pituitrin has been found to inhibit only that which is clearly a water diuresis. We regard our experiments as decisive evidence

that salt diuresis is not due to the same sort of blood dilution that water diuresis is due to.

EXCRETION OF SOLUTES. The present data show that the concentrations in which the diuretic solute is excreted during salt diuresis is uninfluenced by pituitrin. In all cases the concentration mounts higher and higher throughout an experiment, until, when salt diuresis is subsiding, a plateau is reached. The fact that the maximum is reached gradually, whether water diuresis is inhibited by pituitrin or whether there is no excess of water in the body, indicates that this progressive increase in the intensity of solute concentration by the kidneys is an integral part of the salt diuresis; the complete process is not divided or modified by any means now known. The same maximum was attained in equal times in all KCl experiments which were unaccompanied by water diuresis.

In none of our experiments was it certain that the rate of excretion of solutes was significantly influenced by pituitrin. Since water was appropriated by the kidneys for salt diuresis independently of the presence of pituitrin, this is rather to be expected. Table 1 shows slight diminutions in maximal rates of solute excretion in every case where pituitrin was administered; this difference in solute excretion is of an amount which regularly goes with the "washing out" action of water.[6] Comparison of the several curves of solute excretion in figures 3 and 4 shows no differences which fall outside the rather wide limits of experimental variation.

By making use of the excretory ratio, Addis (1918, 1923) has shown that pituitrin markedly lowers the rate of urea excretion both in anesthetized rabbits and in normal men. This evidence, based on blood analysis, is more certain than the comparison of the rates of elimination in separate experiments. It seems probable from the experiments of Addis that pituitrin lowers the sensitiveness of the kidneys to plasma urea in a similar way to its lowering of the threshold for stimulation by water. Addis' experiments allow, however, of the possibility that the urea excretion is temporarily inhibited merely by the accompanying decrease in urinary volume, for a high rate of water excretion is required for the maintenance of Addis' excretory ratio for urea. An increase in the rate of chloride excretion through the agency of pituitrin was reported by Von der Velden (1913) and others.[7]

THE EVERYDAY INFLUENCE OF PITUITRIN. Since pituitrin is continually present in the blood, it has been suggested that it acts as a direct regulator of some of the kidney's activities. Particularly in relation to water excretion has this possibility been emphasized, by Oehme (1921), Starling

[6] For the influence of water on solute excretion see Marshall (1920) (1926), and MacKay and MacKay (1924).

[7] Priestley (1921), Fromherz (1923), Starling and Verney (1925) and Stehle and Bourne (1926).

and Verney (1925), Molitor and Pick (1925), Brown and Rowntree (1925) and Siebeck (1926). The basis for these suggestions rests on the fact that water excretion often seems to bear little relationship to blood dilution.

Now, it has been pointed out above that we do not know what physical measurement of dilution is to be relied upon, and that increased water excretion may be caused by other factors than dilution of plasma. The measurement of dilution has been found to be unexpectedly difficult (Priestley, 1921), and it seems that many factors which are recognized qualitatively have yet to be evaluated before additional factors need be called upon to account for variations. Certainly under most conditions the influence of pituitrin in the blood will be not only constant hour after hour (Geiling, 1926) but powerless to influence the volume of the already highly concentrated urine.

The one experimental evidence for hormone control of kidney activity is found in the observations of Cow (1912). He injected water under the skin and found no diuresis unless a stomach extract accompanied the water. Apparently this experiment has not been repeated (Marshall, 1926). The lack of response to water injections in animals is hardly surprising,[8] for any substance dissolved in the water will prevent the local anesthetic effect of water, and possibly also the antidiuretic effect.

Pituitrin itself has served to emphasize the immense difference which sometimes exists between the kidneys of individuals under various conditions, particularly of anesthesia, decerebration and evisceration.

SUMMARY

1. Pituitrin, injected intramuscularly in men at the same time that various solutions were taken by mouth, did not inhibit the excretion of solutes.

2. Pituitrin inhibited completely the extra excretion of water following ingestion of pure water, but not the extra excretion of water following the ingestion of salts in solutions more concentrated than the maximal excretory concentration.

3. When water and salt (KCl, urea) were taken in solutions isotonic with blood plasma, the presence of pituitrin allowed the excretion of the amount of water which was required for salt diuresis, the remainder was retained.

4. Pituitrin renders the kidneys insensitive to an excess of water in the blood plasma. This fact helps to distinguish diuretic influences due to dilution from those due to salt excretion.

[8] See the experiments of Thompson (1900), of Hashimoto (1914) and of Davis (1917).

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ESTIMATIONS OF AFFERENT ARTERIOLE AND GLOMERULAR CAPILLARY PRESSURES IN THE FROG KIDNEY

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Accurate information concerning the blood pressure in the glomerular capillaries is essential for a satisfactory understanding of the processes which take place there. The modern theory of renal secretion elaborated by Cushny (1917) from the Ludwig filtration-reabsorption hypothesis, demands that during the formation of urine the blood pressure in the glomerular capillaries shall be greater than the osmotic pressure of the plasma colloids. If this is not the case, filtration as we now understand it, is insufficient to account for the separation of a protein-free fluid from the blood in the Malpighian bodies.

The close correspondence in mammals between the osmotic pressure of the plasma proteins, the blood pressure at which secretion of urine ceases, and the difference between blood pressure and the maximum height to which pressure in an occluded ureter may rise, suggest that the formation of urine continues only as long as the pressure in the kidney vessels is higher than the osmotic resistance of the plasma colloids. These blood pressures are carotid pressures, and it is reasonable to question whether they may be taken as a measure of the pressure in the glomerular capillaries. Although the relationships mentioned are not materially altered if the glomerular capillary pressure is taken as 20 per cent below carotid pressure, as is commonly done, this is obviously a surmise.

Direct measurements of glomerular capillary pressures are not yet available. Indirect measurements of the pressure in the glomerular capillaries of the frog's kidney, however, have been made by Hill and McQueen (1921). They compressed a portion of the frog's kidney between a glass plate and a membrane with an apparatus similar to that devised by Roy and Graham-Brown (1880). When external pressure was raised to 5 to 10 mm. mercury, distinct retardation of blood flow in the glomerular capillaries was observed. They imply that this figure is an expression of the glomerular capillary pressure. It must be admitted that retardation of flow in the glomerular capillaries as a criterion of capillary pressure is most difficult of uniform and consistent application. Furthermore, it seems certain that when a small portion of the kidney surface is

submitted to external pressure the veins and venous capillaries will be the first to be compressed, and retardation of blood flow through the glomeruli must occur as soon as any increase in resistance to their venous outflow is imposed, regardless of the pressure within them.

Recent work in this laboratory has shown that the minute structures of the living frog's kidney may be subjected to instrumental manipulation while under direct observation. This has permitted the development of a method for estimating blood pressure in the capillaries and afferent arteriole of a single glomerulus which is similar in principle to the Riva-Rocci method of estimating arterial blood pressure in man. In the work here reported these pressures have been measured and compared with simultaneous measurements of aortic pressure.

METHODS. The frogs (*R. pipiens*) were either pithed or anesthetized with urethane. They were prepared according to the method described by Wearn and Richards (1924) for transillumination of the kidney. Systemic blood pressure was recorded from a cannula in the left aortic arch connected to a Harvard membrane manometer by thick walled rubber tubing. A fine quartz pipette, similar to those used by Wearn and Richards, was sealed into one end of a glass tube, 5.5 cm. long and about 3 mm. bore, bent at an angle of 135°. To this was fused a glass rod which was clamped in a Barbour stage, which in turn was held in a heavy Palmer stativ. The glass tube was connected by thick walled tubing with a Harvard membrane manometer. A T-piece in this tube allowed connection with a tight fitting 2 cc. Luer syringe, and by another branch tube with a leveling bulb. The motion of the syringe plunger was controlled by a screw adjustment. The leveling bulb and its connecting rubber tube was filled with Hg. and the rest of the system with 0.6 per cent NaCl containing indigocarmine and a small amount of sodium taurocholate.[1] The leveling bulb was used only for filling, washing the pipette, and for gross adjustments, and was clamped off while pressure measurements were being made. Both manometers were calibrated against a column of water before and after each experiment. A diagram of the apparatus is shown in figure 1.

After the pipette had been introduced into Bowman's space, a minute amount of colored fluid was injected by means of the syringe in order to identify the tubule into which it emptied. A slender, blunt rod, about 40μ in diameter, held in a second Barbour stage was then brought down on one of the proximal loops of this tubule, occluding its lumen by pressure. Complete occlusion was judged by the failure of dye subsequently injected to pass beyond the point of occlusion, and by the appearance of

[1] The sodium taurocholate, suggested by Barcroft's use of it in the differential blood gas manometer, was added to lower surface tension.

blue granules within the lumen of the tubule distal to the point of occlusion which did not move when the intracapsular pressure was varied. (These granules consisted of dye, precipitated by the absorption of fluid from that injected before occluding the tubule.) On removing the rod at the end of the experiment these granules were washed down the tubule by intracapsular pressures which had previously been unable to move them.

A slight intracapsular pressure was sufficient to distend Bowman's capsule. As the intracapsular pressure increased the tuft decreased in size, then blood flow could be seen to cease in some of the capillaries, while continuing in others. Further increase in pressure caused the blood flow through those remaining open to become pulsatile, with distinct slowing during diastole. Then flow stopped completely for part of the cardiac

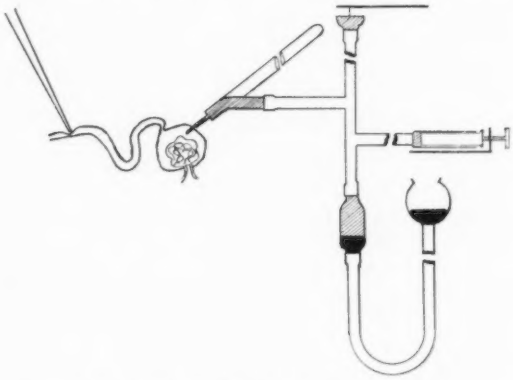

Fig. 1. Diagram of capillary pipette and apparatus for altering and measuring intracapsular pressure.

cycle, the red cells moving onward in the capillaries only during systole. The distance traversed during each systole diminished as the pressure rose, until finally only a few cells entered the tuft with each systole. When the pressure was still further increased, no cells entered the tuft, but pulsations could be seen in the cells in the afferent artery.

In making measurements, equalization of pressure within the capsular space and the manometer system was most advantageously secured by raising the pressure in the manometer system to a point somewhat higher than that sufficient to obliterate all blood flow in the glomerulus; then by lowering the pressure by means of the syringe, first to the point where cells entered the tuft during each systole (afferent arterial pressure), and second, to the point where blood flow was continuous through one or two capillaries of the tuft (glomerular capillary pressure). A record of several estimations is shown in figure 2.

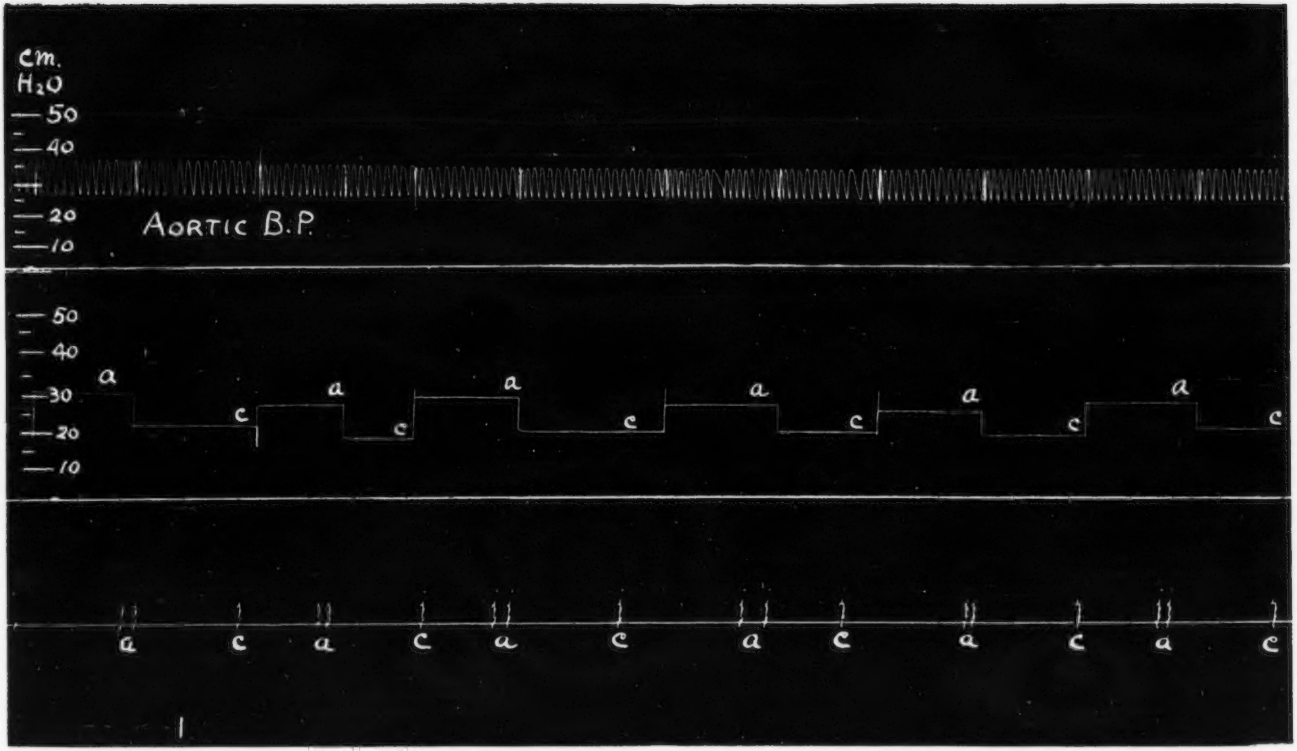

Fig. 2. Upper record = aortic pressure as recorded by a membrane manometer

The second record was made by a capillary pipette inserted into the capsule of Bowman. Estimations of afferent artery pressure, *a*, alternate with estimations of glomerular capillary pressure, *c*. The exact points at which the criteria of these pressures were identified are indicated on the lowest signal line.

The intracapsular pressure at which several corpuscles entered the tuft during each systole was taken as a measure of the systolic pressure in the afferent artery. The minimum intracapsular pressure at which no cells enter the tuft is appreciably higher than the maximal intracapsular pressure at which entrance of cells is permitted. The former obviously represents the pressure within the renal arteriole at the point of origin of the afferent vessel, and hence is slightly higher than the pressure sought. The latter is obviously slightly lower than the systolic pressure in the afferent vessel. It was chosen, however, because of the difficulty frequently met in clearly seeing the afferent artery, and because of the advantage of confining observations to a single structure.

The highest pressure at which blood flow was continuous through at least one capillary of the glomerular tuft was accepted as a measure of capillary pressure, or more exactly, diastolic capillary pressure. According to this criterion, flow through the capillary might be slowed during diastole, but must not stop. If flow continued in only a single capillary, it could be maintained with reason that the pressure recorded was not the normal capillary pressure, but approximately the diastolic pressure in the afferent artery, for under these circumstances there would be an unbranched tube from renal arteriole to efferent vessel. This criticism is applicable to only a few of the measurements recorded as capillary pressure. Most frequently, as the intracapsular pressure was lowered, the corpuscles which entered tuft with each systole separated into two or more paths before flow was continuous in any one. In such cases, pressure at which flow continued in one capillary would be equivalent to that in the one occluded during diastole but open during systole. On still further reducing intracapsular pressure, other capillaries were opened. In other instances, by the time intracapsular pressure was reduced sufficiently to obtain continuous flow through any capillary, all seemed to be flowing about equally. These tufts gave the lower capillary pressure readings. The reason for this difference in the behavior of different tufts is not clear.

CONTROL EXPERIMENTS. A variety of control experiments were made to determine the adequacy of the apparatus and method.

The first of these was designed to give assurance that small changes in pressure were transmitted promptly and completely through such a fine capillary as the pipette (10 to 20μ inside diameter) under conditions which did not permit appreciable transfer of fluid. A capillary pippette was filled with an indigocarmine solution and connected with a manometer and reservoir for varying the pressure within the system. The point was thrust through the thin rubber covering of a water-filled glass capsule also connected with a manometer and reservoir. The capsule rested on the stage of a binocular microscope, which was focussed on the tip of the pipette. When the pressure was considerably higher in the

cated on the lowest signal line.

pipette system, fluid could be seen pouring from the tip of the pipette in a fine blue stream; if the difference was slight, the tip of the pipette appeared capped by a tiny blue globule. When the pressure was higher in the capsule system, the tip of the pipette appeared colorless, the blue being apparent only further up where its capacity was greater. The appearances at the pipette tip were unmistakable. The average difference in the pressures of the two systems required to initiate flow out of or into the tip of the pipette was 0.42 cm. H_2O in 48 readings. The greatest difference was 1.6 cm. H_2O, the least 0.0 cm. H_2O. In 42 of the 48 readings the difference was less than 1 cm. H_2O; in the last 13 consecutive readings (last experiment) the average difference was 0.1 cm. H_2O, the greatest 0.3 cm. H_2O. This was considered evidence that the use of such a fine capillary did not preclude satisfactory pressure measurements.

Next, control estimations were made using a model so constructed that the conditions encountered in work with the glomerulus could be grossly imitated. It consisted of two glass tubes connected by a piece of cat's jugular vein. The tubes projected into a small glass chamber so that the vein was wholly within it. The chamber was provided with a rubber covered window and connected with a manometer and reservoir. It was placed on the stage of a microscope and the point of a capillary pipette thrust through the rubber window. Salt solution was then allowed to flow through the vein at various pressures. Measurements of the minimal pressure in the glass chamber necessary to prevent flow through the vein were made both by the manometer connected directly to the glass chamber and by that connected with the capillary pipette. The difference in the readings of the two manometers averaged less than 0.1 cm. H_2O; greatest difference 0.2 cm. H_2O (13 observations). The difference between the pressure head of fluid flowing through the vein and the pressure required to prevent flow averaged less than 0.1 cm. H_2O; greatest difference 0.6 cm. H_2O (29 observations).

As a further control of the method, estimations were made of the afferent arteriole and capillary pressures in six perfused kidneys. Large specimens of *R. catesbiana* were perfused with oxygenated Hamburger's solution through a cannula in one branch of the aorta. The coeliaco-mesenteric artery and the aorta below the renals were tied. A side arm on the cannula was connected with the Harvard manometer for recording perfusion pressure. Washed frog corpuscles were added to the perfusion fluid to indicate the presence or absence of flow through the capillaries of tuft. Since perfusion pressure was constant the criteria for afferent artery and capillary pressures discussed above had to be modified. The pressure at which flow through some capillary loops ceased, while persisting in at least one, was regarded as a measure of capillary pressure;

TABLE 1

Measurements of aortic, afferent artery, and glomerular capillary pressures in perfused kidneys

IN CENTIMETERS OF H_2O			AS PERCENTAGE OF PERFUSION PRESSURE	
Perfusion pressure	Afferent artery	Glomerular capillaries	Afferent artery	Glomerular capillaries
22	20	10	91	46
	21	10	95	46
	26	20	118	91
	22	16	100	73
	18	13	82	59
	20	15	91	68
	23	15	104	68
23	22	5	95	22
24	22	13	91	54
	17	6	65	23
26	20	11	72	42
31	27	20	87	64
	28	18	90	53
	25	16	80	52
33	26	17	79	52
	30	17	91	52
	26	17	79	52
	26	17	79	52
	26	15	79	45
	19	17	57	52
		17		52
34	22	5	64	14
35	40	26	114	74
	30	13	81	37
	27	20	72	57
	28	18	80	51
38	33	30	87	79
	36	24	94	63
	33	22	87	58
43	35	28	81	65
	50	27	116	63
	42	38	97	83
	42	30	97	69
	33	25	77	58
		19		44

TABLE 1—*Concluded*

IN CENTIMETERS OF H_2O			AS PERCENTAGE OF PERFUSION PRESSURE	
Perfusion pressure	Afferent artery	Glomerular capillaries	Afferent artery	Glomerular capillaries
44	35	24	79	55
	38	26	81	60
	33	17	75	38
45	47	26	104	58
46	40	24	82	52
	42	23	91	50
	41	21	90	45

minimal pressure at which flow through all the capillaries of the tuft ceased as a measure of afferent arteriole pressure.

Perfusion pressure was varied between 22 and 46 cm. H_2O. Afferent artery pressure averaged 87 per cent (86.8 per cent) of perfusion pressure in 40 observations. Individual readings varied from 17 to 50 cm. H_2O, or from 64 to 118 per cent of the perfusion pressure; 26 of the 40 observations being between 75 to 95 per cent of perfusion pressure. Capillary pressure averaged 52 per cent of perfusion pressure in 42 observations. Readings varied from 5 to 38 cm. H_2O, or 14 to 91 per cent of perfusion pressure; 25 of the 42 readings being between 40 to 60 per cent of perfusion pressure (table 1).

EXPERIMENTAL DATA. The data presented here represent over 190 measurements on 18 frogs, varying in weight from about 50 to 125 grams. In the great majority of measurements, no attempt was made to alter artificially conditions within the renal circulation. The few instances in which such alterations were attempted are discussed separately. Salt solution (0.6 per cent), however, was injected into the anterior abdominal vein in amounts of 0.1 to 0.3 cc. when needed to maintain the circulation. In some experiments 3 per cent acacia was substituted for the salt solution. In most instances an estimation of afferent artery pressure was followed immediately by one of capillary pressure, in order to permit comparison of aortic, arteriole and capillary pressures recorded under conditions as nearly identical as possible. Thus the relation of systolic afferent artery to systolic aortic pressure is based on 186 observations; capillary pressure to systolic aortic pressure, 181 observations; capillary pressure to systolic afferent artery pressure, 168 observations.

Systolic aortic pressure measurements varied between 21 and 61 cm. H_2O, most frequently lying between 25 to 44 cm. H_2O (during 138 of the 186 observations). The mean of the measurements of systemic aortic

pressure during the observations of afferent artery pressure was 37.2 ± 6.4 cm. H_2O, its standard deviation 9.7 cm. H_2O.

Systolic afferent artery pressure varied between 15 and 56 cm. H_2O, most frequently lying between 16 and 39 cm. H_2O (144 of the 186 observations). The mean of the estimations of systolic afferent artery pressures was 31.6 ± 6.0 cm. H_2O, its standard deviation 9.1 cm. H_2O. The coefficient of correlation (r) between systolic afferent artery and systolic aortic

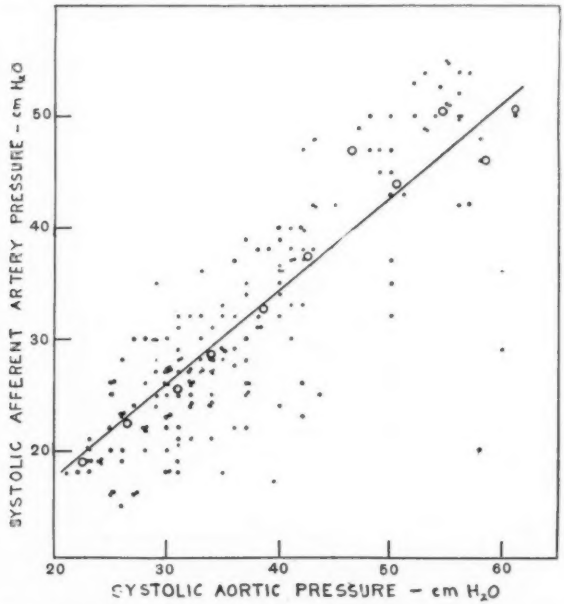

Chart 1. Relation of systolic pressures in the afferent artery and the aorta. Dots represent single measurements of pressure in the afferent artery; circles represent group averages, each group including all measurements within a 4 cm. rise in aortic pressure. Graph was drawn from group averages; its equation, $y = 3.37\ x + 16.3$.

pressures was 0.93 ± 0.01. This was calculated without using Sheppard's correction.

Expressed in another way, afferent artery pressure averaged 85 per cent of the simultaneous systolic aortic pressure, individual estimations ranging from 35 per cent to 112 per cent, while in 112 (60 per cent) of the estimations the spread was 73 to 93 per cent.

Estimations of capillary diastolic pressure ranged from 4 to 52 cm. H_2O, most frequently lying between 10 and 27 cm. H_2O (141 of 181 estimations). Only 11 or 6 per cent of the estimations were less than 10 cm.

H_2O. The mean of the estimations of capillary pressure was 20.2 ± 6.8 cm. H_2O, its standard deviation 10.2 cm. H_2O. (The mean of estimations of systolic aortic pressure at the time of capillary pressure estimations was 37.4 ± 6.7 cm. H_2O, the slight difference between this and the mean formerly given being due to the few instances in which afferent artery and capillary pressure estimations were made independently of each other.) The coefficient of correlation (r) between systolic aortic

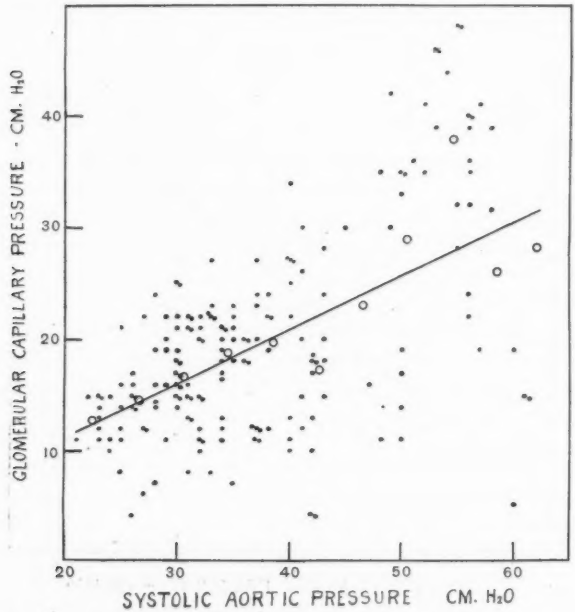

Chart 2. Relation of systolic pressure in the aorta and pressure in the glomerular capillaries. Dots represent single measurements; circles represent group averages, each group including all measurements within a 4 cm. rise in aortic pressure. Graph was drawn from group averages; its equation, $y = 1.89\,x + 10.9$.

and diastolic capillary pressure was 0.54 ± 0.03. The mean of the estimations of diastolic capillary pressure was 54 per cent of the mean of the estimations of systolic aortic pressure; individual estimations varied from 8 to 87 per cent of aortic pressure, being between 41 and 70 per cent in 111 instances.

Paired measurements of afferent artery and capillary pressures were made in 168 instances. Here the mean of the estimations of systolic afferent artery pressure was 33.0 ± 6.9 cm. H_2O and the mean of esti-

mations of diastolic capillary pressure 21.0 ± 6 cm. H_2O, the coefficient of correlation (r) 0.69 ± 0.03. The mean capillary pressure was 64 per cent of afferent artery pressure.

Individual estimations ranged from 14 to 92 per cent of the systolic afferent artery pressure. In 109 of 168 observations the range was from 50 to 80 per cent (table 2).

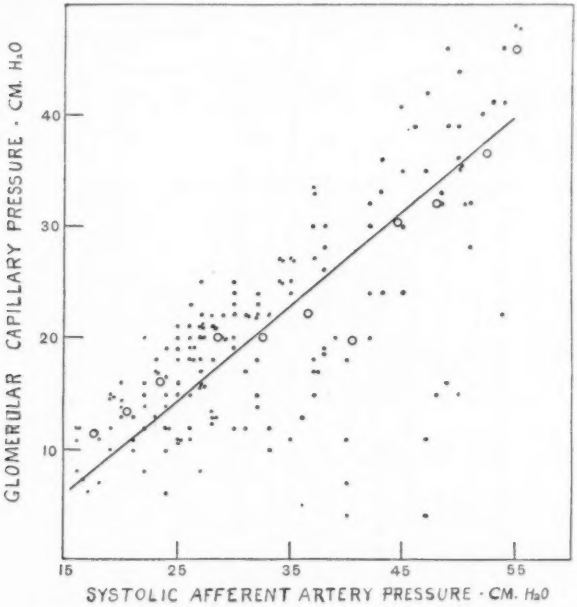

Chart 3. Relation of systolic pressure in the afferent artery and pressure in the glomerular capillaries. Dots represent single measurements; circles represent group averages, each group including all measurements within a 4 cm. rise in pressure in the afferent artery. Graph drawn from group averages; its equation, $y = 3.38 x + 4.1$.

ACTION OF ADRENALIN AND CAFFEINE. A few observations of the effect of varying doses of adrenalin and caffeine on afferent artery and glomerular capillary pressure have been made. The conception that intraglomerular pressure may be regulated in part by differences in the degree of constriction of afferent and efferent arteries is implied in a statement of Ludwig's in 1856. It was stated more clearly by Starling (1920) but no experimental evidence was available until the work of Richards and Plant in 1922. Additional support is to be found in the experiments of Menden-

TABLE 2

Measurements of aortic, afferent artery and glomerular capillary pressures in living frogs

FROG NUMBER	IN CENTIMETERS OF WATER			AS PERCENTAGE OF AORTIC PRESSURE		FROG NUMBER	IN CENTIMETERS OF WATER			AS PERCENTAGE OF AORTIC PRESSURE	
	Systolic aortic pressure	Systolic afferent artery pressure	Glomerular capillary pressure	Systolic afferent artery	Glomerular capillary		Systolic aortic pressure	Systolic afferent artery pressure	Glomerular capillary pressure	Systolic afferent artery	Glomerular capillary
18	21	18	11	86	52	7	28	30	24	107	86
						16		22	7	78	25
5	22	18	15	82	68	18		26	19	93	68
18	23	19	15	82	65	5	29	28	22	96	76
18		19	12	82	52	5		24	11	83	38
18		20	13	87	56	7		30	19	103	65
18		18	11	78	48	7		25	20	86	69
18		21	15	91	65	7		27	16	93	55
18		20	14	87	61						
						1	30	20	14	67	47
5	24	19	15	79	62	1		18	16	60	53
18		19	10	79	42	1		18	25	60	83
18		19	11	79	46	7		27	22	90	73
						7		27	17	90	57
1	25	16	11	64	44	7		23	18	72	60
15		26		104		7		23	15	72	50
16		16	8	64	32	7		25	25	83	83
18		25	16	100	64	7		30	21	100	70
18		26	21	104	84	7		27		90	
18		20	14	80	56	8		26	19	86	63
18		22	12	88	48	15		27	17	90	55
						15		27	16	90	53
1	26	19	15	73	58	18		26	18	86	60
5		28	15	108	58	18		22	15	73	50
16		15	4	53	15						
18		23	17	88	65	1	31	32	15	103	48
18		23	14	88	54	1		18	15	58	48
18		20	16	77	61	1		19	13	61	42
18		23	14	88	54	1		21		67	
						1		21		67	
1	27	16	12	59	44	7		25	21	81	67
1		16	12	59	44	7		31	22	100	71
2		30	22	112	81	8		28	21	90	67
15		24	6	89	22	15		28	13	90	42
						15		22	8	71	26
1	28	22	16	78	57	15		27	16	87	51
1		20	14	71	52	18		29	22	93	71
2		22	14	78	50						

TABLE 2—*Continued*

FROG NUMBER	IN CENTIMETERS OF WATER			AS PERCENTAGE OF AORTIC PRESSURE		FROG NUMBER	IN CENTIMETERS OF WATER			AS PERCENTAGE OF AORTIC PRESSURE	
	Systolic aortic pressure	Systolic afferent artery pressure	Glomerular capillary pressure	Systolic afferent artery	Glomerular capillary		Systolic aortic pressure	Systolic afferent artery pressure	Glomerular capillary pressure	Systolic afferent artery	Glomerular capillary
1	32	24	15	75	47	2	37	34	27	92	73
2		25	11	78	34	2		25	11	62	30
2		27	20	84	62	2		25	11	62	30
2		26	11	81	34	2		22	12	59	32
2		24	12	75	37	2		26	20	70	54
7		26	20	81	62	8		37	24	100	65
8		26	15	81	47	8		35		94	
16		32	22	100	68	8		26		70	
17		27	21	84	65	13		30	23	81	62
18		21	10	65	31	13		28	12	76	32
						13			12		32
8	33	32	22	97	66						
8		28	22	85	66	5	38	31	12	81	32
8		27	22	82	66	5		32	24	84	63
8		30	23	91	69	5		31	22	81	58
16		27	8	82	24	13		38	19	100	50
16		36	27	109	82	13			22		58
2	34	24	16	70	48	1	39	38		97	
2		28	13	82	38						
2		24	12	70	35	1	40	36	13	90	32
2		27	18	79	53	2		32	22	80	55
5		27	20	79	59	3		40	11	100	27
5		25	19	73	56	3		34	10	60	25
5		27	21	79	61	5		34	27	85	62
8		23	21	67	61	5		39	34	97	85
8		28		82		17		35	25	87	62
16		31	17	91	50	17		36	27	90	67
18		21	11	62	32	17		36	27	90	67
2	35	33	22	94	63	3	41	33	12	80	29
2		29	21	83	60	3		37	15	90	36
5		28	18	80	51	3		40	7	98	17
5		29	20	83	57	5		38	26	93	63
18		18	7	51	20	5		37	30	90	73
18		32	23	91	66	9		30	20	73	49
8	36	29	20	80	55	3	42	40	18	95	43
8		32	18	89	50	3		47	4	112	9
8		22	20	61	55	3		37	17	88	40
13		27	18	76	50	3		40	4	95	9
13		37		103		3		33	10	78	24

TABLE 2—*Concluded*

FROG NUMBER	IN CENTIMETERS OF WATER			AS PERCENTAGE OF AORTIC PRESSURE		FROG NUMBER	IN CENTIMETERS OF WATER			AS PERCENTAGE OF AORTIC PRESSURE	
	Systolic aortic pressure	Systolic afferent artery pressure	Glomerular capillary pressure	Systolic afferent artery	Glomerular capillary		Systolic aortic pressure	Systolic afferent artery pressure	Glomerular capillary pressure	Systolic afferent artery	Glomerular capillary
9		38	18	90	44	6	53	49	39	92	73
9		26	18	60	43	6		49	46	92	87
9		23	13	54	31	6		54	46	102	87
3	43	38	28	88	65	6	54	50	44	92	81
3		42	24	97	56						
3		42	20	97	46	6	55	55	48	100	87
3		48	15	112	35	6		55	48	100	87
10		25	18	57	42	11		51	32	92	58
						11		51	28	92	51
9	45	42	30	93	66						
						11	56	54	22	96	39
4	47	49	16	104	34	11		42	32	75	52
						11		50	39	89	69
4	48	50	35	104	73	11		52	24	92	43
4		47	11	98	23	11		50	40	89	71
						11		50	40	89	71
4	49	45	30	92	61	11			35		62
6		47	42	96	81	11			36		64
4	50	50	19	100	38	11	57	54	41	95	72
6		43	33	86	66	12		43	19	71	33
6		45	35	90	70						
6		47	35	94	70	12	58	20		34	
14		32	14	64	28	12		48	32	83	55
14		37	17	74	34	12		46	39	79	67
14		35	11	70	22						
						12	60	24	19	40	32
6	51	43	36	84	70	12		36	5	60	8
6	52	53	41	102	79	12	61	50	15	82	24
11		50	35	96	62	12			15		24

hall, Taylor and Richards (1924), Richards and Schmidt (1924), and Richards, Barnwell, and Bradley (1926).

Adrenalin, in concentrations of 1:250,000 to 1:500,000, was either injected into the anterior abdominal vein (0.1 to 0.2 cc.) or dropped directly on the surface of the kidney. Caffeine, 1 or 2 per cent, was injected intravenously in amounts between 0.05 and 0.2 cc. The estimations are too few in number to treat statistically. This is unfortunate, because

estimations, particularly of capillary pressure, varied so much in consecutive readings that such treatment is desirable. The opportunities for satisfactory estimations of differential effects on afferent and efferent vessel were few. When too large a dose of adrenalin was used, the circulation through the glomerulus was stopped entirely; when the dose was very small, the changes were within those of subsequent readings. Moreover, it was difficult to make estimations in rapid succession, because when the capillaries were not allowed to expand and "rest" between readings, their response to changes in intracapsular pressure became sluggish and unsatisfactory. Again, it was considered proper to inject a drug only after several readings in succession had given closely agreeing values. The observations accepted, however, were all made in experiments in which the preparation and control readings were satisfactory. While therefore the actual values given have not the reliability of the averages of larger numbers of estimations, they are regarded as trustworthy evidence that intraglomerular pressure may at times be markedly influenced by other factors than systemic blood pressure. Intravenous injections of salt solution in similar amounts raised aortic pressure, and with it generally afferent artery and glomerular capillary pressures, but did not alter the existing relations between them. These measurements are presented in table 3.

Discussion. The application of the Riva-Rocci principle to the estimation of glomerular capillary pressure and the results obtained by it require some further comment. The usefulness of the Riva-Rocci method for estimation of pressures in large vessels has been established; its use in connection with vessels of capillary size may be questioned. The one factor which might operate to vitiate the results in this use is the viscosity of blood which is so largely due to its content of corpuscles. Movement of corpuscles and not transmission of pulse was our criterion of pressures. Evidence exists which shows that the passage of corpuscles through capillaries may be interrupted under some conditions which permit passage of plasma. It is therefore possible that the pressure at which visible blood flow through several capillaries of the glomerulus ceases is not the pressure at which all flow of plasma ceases. If such were the case the pressure reading would be too low.

Differences in rate and volume of blood flow within the capillaries of a single glomerulus appear to depend not so much upon differences in calibre of the capillaries themselves as upon differences in diameter of the capillary openings from the afferent vessel. If, among the three primary divisions, the openings of two were narrow and the third wide, it is clear that the pressure required to obliterate visible flow in the two might be much lower than that required for the third. Error arising from such a source as this would also make the estimate low. In selecting the

Effect of adrenalin, caffeine and salt solution on aortic, afferent artery, and glomerular capillary pressures

FROG NUMBER	MEASUREMENTS OF			PERCENTAGE CHANGES IN PRESSURE IN			REMARKS
	Systolic aortic pressure	Systolic afferent artery pressure	Glomerular capillary pressure	Aorta	Afferent artery	Capillaries	
	cm. H_2O	cm. H_2O	cm. H_2O				
2	Before 37, 37, 37 After 40, 35	25, 22, 26 32, 29	11, 12 22, 21	+8.1	+23.0	+83.5	Adrenalin 1:500,000 (old) intravenously
5	Before 38, 38 After 43, 43, 41	32, 31 46, 42, 38	24, 22 36, 37, 30	+13.0	+48.5	+63.5	Adrenalin 1:250,000 (old) intravenously
16	Before 25 After 30, 37 Later 28, 28	16 17, 18 18	8 12, 0* 7	+23	+12.5	+50.0	Adrenalin 1:100,000, 2 drops in body cavity
17	Before 32, 32 After 34, 35	34, 34 33, 32	25, 25 22, 23	+9.4	−5.9	−12.0	Adrenalin 1:500,000, 2 drops on kidney. Caffeine previously
5	Before 41, 41 After 46, 45, 43 Later 40	37, 37 56, 51, 53 39	33, 33 46, 45, 40 34	+12.0	+51.4	+39.4	Adrenalin, about 1:500,000, intravenously
18	Before 25, 25 After 26	25, 26 31	16, 21 26	+4.0	+24	+62.5	Adrenalin 1:500,000, 2 drops on kidney
18	Before 26, 23 After 23, 23, 23	23, 20 18, 21, 20	14, 13 11, 15, 14	0	0	+7.0	Adrenalin 1:500,000, 2 drops on kidney
18	Before 24, 23, 21 After 21, 25, 24 Later 24	19, 19, 18 18, 16, 19 19	10, 15, 11 0*, 6, 8 11	+8.0	−15.0	−36	Adrenalin 1:500,000, 2 drops on kidney

13	Before 36, 38	37, 38	18, 19				Caffeine, 0.2 cc. 1 per cent intravenously
	After 37	50	35	0	+35	+84	
15	Before 30, 30	27, 27	17, 16				Caffeine, 0.2 cc. 1 per cent intravenously
	After 30	37	21	0	+37	+31	
16	Before 33, 32	36, 32	27, 22				Caffeine, 0.4 cc. 1 per cent intravenously
	After 25, 23	17, 16	7, 6	−28	−55	−70	
17	Before 40, 40	35, 36	25, 27				Caffeine, 0.4 cc. 2 per cent intravenously
	After 32, 30	27, 28	21, 20	−25	−25	−20	
18	Before 34, 32	21, 21	11, 10				Caffeine, 0.2 cc. 2 per cent intravenously
	After 25	25	17	−22	+19	+54	
18	Before 35, 31, 30	32, 29, 32	23, 22, 25				Caffeine, 0.2 cc. 2 per cent intravenously
	After 28, 26, 25	31, 23, 20	21, 14, 14	−16	−37	−44	
18	Before 30, 28	26, 26	18, 19				Caffeine, 0.2 cc. 2 per cent intravenously
	After 28, 27	30, 27	22, 17	−3.5	+15	+15	
	Later 25	22	12				
2	Before 37, 37	25, 22	9, 10				NaCl 0.6 per cent, 0.4 cc. intravenously
	After 37	26	8	0	+18	+20	
5	Before 34, 34	25, 27	19, 21				NaCl 0.6 per cent, 0.2 cc. intravenously
	After 38, 38	32, 31	24, 22	+11	+18	+14	
7	Before 30, 30	27, 27	22, 22				NaCl 0.6 per cent, 0.2 cc. intravenously
	After 32, 30	26, 23	17, 18	+6	−3	−22	

* No flow through glomerulus due to constriction of afferent artery.

glomerulus for study in any experiment, thought was given to the matter of uniformity of blood flow through the entire tuft, none being selected in which obvious differences existed among its several capillaries.

It is to be emphasized that in these measurements no effort was made to estimate the pressure within a single capillary: the desired value was one representing the true order of magnitude of the average pressure in the group of vessels which make up the glomerulus. It is conceded that pressure may not be uniform within the different capillaries of one tuft; that average pressure may differ among various glomeruli: but the consistency of the results cited, together with the conscious efforts made to select suitable capsules for the estimations, gives strong indication that no significant variable factor of error was involved.

The relative elasticity of the capsule of Bowman in comparison with that of a suitable sphygmomanometer cuff probably introduces a slight error due to the transmission of pulsations outside the system. This error would tend to give too high readings; but in these measurements it must be much smaller than when arterial pressures are measured, because at this level of the vascular system pulse pressure is not so conspicuous.

One factor cannot be evaluated without further anatomical studies of an exquisite sort, i.e., the effect of distention of the capsule of Bowman on the capillary openings. If by stretching the capsule the orifices of the capillaries were widened the conditions of measurement would be distorted in comparison with normal, and false values would be obtained. Until we actually know the shape and arrangement of these orifices in relation to the attachment of the capsule to the afferent vessel we have little basis even for speculation concerning this factor. The point is mentioned as a possible source of future correction for this work.

Our control experiments have revealed no flaw resident in the use of a fine capillary tube for the transmission of intracapsular pressure to the measuring system.

One point of technique needs comment for the sake of those who have not had experience with this type of experimentation. When puncture of the capsule of Bowman is made with nicety, without lateral to and fro motion of the pipette, a clean hole is made in the capsule, the edges of which adhere to the outer wall of the pipette. The sealing of the pipette into this hole is further strengthened by the clotted lymph which accumulates on the surface of the kidney. At the end of a good experiment, when the pipette is withdrawn, the capsule of Bowman is seen to be pulled with the pipette to such an extent as to indicate that the seal is capable of withstanding considerable tension. In every accepted experiment of the group reported here actual tests were introduced—indeed the method itself includes such—which guaranteed the tightness of the space in which pressure was being measured. Pressure was raised by injection of colored

fluid and was maintained sufficiently long to give assurance that there was no visible leakage of the fluid either along the tubule beyond the point of obstruction or out into the tissue space surrounding the capsule. The distended condition of the capsule and the contracted condition of the tuft persisted without recognizable change during a period longer than was required for an actual measurement.

We regard the capillary pressures read as diastolic pressures in the capillary system and have given reasons for so doing. By mere inspection one can see that under certain conditions pulse pressure exists within the glomerular capillaries, the whole tuft swelling and shrinking with systole and diastole of the heart. The conditions in which this is most apparent are those in which the circulation is depleted or blood pressure low from other causes. On the other hand, when the circulatory condition of the animal is good, blood pressure high, heart and vessels well filled, and no evidence of arterial constriction in the kidney, visible pulsations in the tuft and visible variations in velocity of blood movement through the capillaries are absent. That this does not necessarily indicate obliteration of pulse pressure is shown by Hürthle's (1923) observations on mesenteric capillaries. His photographic method revealed pulsatile flow which the eye could not recognize. On the other hand, in a number of instances the same method revealed only uniform, continuous flow. Whether the glomerulus which is functioning under optimal conditions is perfused with a pulsatile or constant flow must remain an open question so far as these observations go.

It is striking that in both the perfused and the normal frogs' kidneys, under a wide range of aortic pressures, the value of the glomerular capillary pressure as here measured is so constant at between 40 and 70 per cent of the aortic pressure, while the afferent artery pressure is 70–90 per cent of the aortic pressure. In the microscopically short distance between afferent artery and glomerular capillaries an abrupt fall in pressure occurs, for the explanation of which something more is needed than the increment of resistance furnished by a uniformly narrowing tube. A uniformly narrowing tube does not exist among the vessels which concern us; on the contrary, the afferent vessel breaks up into an assemblage of vessels, the diameter of any one of which may be seen to be greater than that of the afferent vessel. This phenomenon, evaluated as critically as we are able, seems to us to point to an abrupt interposition of resistance at the point of entrance of the afferent vessel into the capsule, or at the point of division of the afferent vessel. Richards and Schmidt (1924) described phenomena which yielded direct objective evidence pointing to the same conclusion.

The glomerular capillary pressure as measured by us is greater than the capillary pressures in other vascular areas of the frog as measured by

Landis (1926). That this must be so was suggested immediately after the relation of the glomerular capillaries to the afferent and efferent vessel and the second system of capillaries had been identified. It is satisfying to find the suggestion upheld by direct evidence.

The estimations in which afferent artery pressure was found to be higher than systolic aortic pressure during the action of adrenalin and caffeine, while open to question, may well come under the "differential pressures" discussed by Bazett (1924). Under certain conditions, especially those obtaining in aortic regurgitation, the systolic pressure is higher in the femoral artery than in the carotid. This Bazett believes to be due to the presence of pressure waves reflected from the periphery. This differential pressure is increased during splanchnic stimulation (fig. 14, p. 571 of Bazett's paper), when it is probable that constriction is taking place in the smaller arterioles, and since such reflected pressure waves are dissipated as they progress, it is highly probable that their effect is greater in the smaller vessels than in the large. Burdick, Clark, Garlichs, Priestley and Richards (1925) found systolic pressure in the femoral artery in man constantly higher than in the brachial, and that the difference was increased after exercise. Here again the point of reflection, and theoretically of maximum pressure, is peripheral to the point of measurement.

These results have obvious bearing upon the filtration theory of urine formation, since they furnish evidence in support of the suppositions which were made by the originator of the theory and accepted by all of its subsequent adherents. Only 11 out of 181 measurements of glomerular capillary pressure fall below 10 cm. of H_2O, the average figure which White (1924) found to represent the osmotic pressure of the proteins of frog's plasma. The results of the study by Hill and McQueen were lower than this; we have indicated possible criticisms of their method and it is to be noted that in their rejection of the filtration theory they did not take account of the fact that the osmotic pressure of frog's plasma proteins is less than half of that of the proteins in mammalian plasma.

SUMMARY

A method has been developed for altering and measuring the pressure within a capsule of Bowman in the frog's kidney which has made possible the estimation of blood pressure in the glomerular capillaries and in the afferent artery by an application of the Riva-Rocci principle. Over 190 such estimations have been made during simultaneous record of aortic pressure.

Systolic pressures in the aorta ranged from 21 to 61 cm. H_2O; in the afferent artery, 15 to 56 cm. H_2O; glomerular capillary pressure varied between 4 to 52 cm. H_2O.

Systolic pressure in the afferent artery averaged 85 per cent of simultaneous aortic pressure; pressure in the glomerular capillaries, 54 per cent.

In general there is a high degree of direct correlation between systolic aortic and systolic afferent artery pressures, between systolic aortic and capillary, and between systolic afferent artery and capillary pressures.

In a few experiments in which the action of adrenalin and caffeine were tested, results were obtained which agree with the view that a substance by action on either afferent or efferent vessel may influence glomerular capillary pressure to a degree which is quite out of proportion to its effect on aortic pressure. Under these circumstances the correlation between aortic and capillary pressure is in abeyance.

I am indebted to Doctor Richards for the suggestion which led to this work and for invaluable aid throughout its course and in the preparation of the manuscript.

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THE EFFECT OF SMALL AMOUNTS OF ADRENALIN UPON THE GLOMERULAR BLOOD VESSELS OF THE FROG'S KIDNEY PERFUSED AT CONSTANT RATE

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It has been shown by Richards and Plant (1922) that when the rabbit's kidney is perfused *in situ* with whole blood, under circumstances which insure constancy of blood flow through the organ regardless of changes in the state of its vessels, the addition of small amounts of adrenalin to the perfusing blood causes perfusion pressure to rise, kidney volume to increase, and urine output to increase. These observations which point to coincidence of vasoconstriction and vasodilatation in the same organ were regarded as evidence that the efferent vessel from the glomerulus as well as the afferent glomerular arteriole is subject to the constricting effect of adrenalin; and that consequently under the conditions of those experiments pressure within the intervening glomerular capillaries increased and caused their distention to increase. In other experiments on eviscerated rabbits, the intravenous injection of very minute amounts of adrenalin caused blood flow through the kidney to decrease, kidney volume to swell, and urine output to increase: and similar results were obtained when pituitrin or barium chloride in minute dosage was substituted for adrenalin (Richards and Plant, 1922; Mendenhall, Taylor and Richards, 1924). In these latter experiments it was thought that increase in resistance to blood flow through the kidney was caused by constrictor action exerted more effectively upon the efferent than upon the afferent vessels, probably because of the smaller calibre of the former.

For the purpose of gaining more convincing evidence upon this question the method of direct microscopic examination of the living frog's kidney was introduced by Richards and Schmidt (1924): and it was found that the intravenous injection of extremely small dosages of adrenalin caused the glomerular tuft to swell precisely as though the efferent vessel were slightly narrowed. But it was recognized that such a result might be produced by other causes than that suggested and final decision was reserved until the action of adrenalin could be tested in the frog's kidney,

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exposed for microscopic examination of the glomerular vessels, and perfused under conditions which did not permit changes in blood flow through it.

After a number of futile efforts, a simple method has been developed for the perfusion of the frog's kidney with whole blood at constant rate of flow; and it is the purpose of this paper to describe experiments in which, by means of it, controlled conditions have been secured under which the size of the glomerular tufts has been studied. The results support the view that adrenalin causes constriction of vessels distal to the glomerulus.

Fig. 1. Apparatus for perfusing the frog's kidney at constant rate of blood flow

METHODS. The perfusion apparatus is pictured in figure 1. It consists essentially of two 5 cc. Record syringes of identical calibre mounted on a board by two brass supports. The nozzle of each syringe projects through a hole in one end of its support: a threaded sleeve passes through the other end, and through this in turn passes a long screw, one end of which carries a cap to fit over the end of the piston of the syringe; the other is fitted with a

crank. The pitch of the screw is 42 threads to the inch and 63 complete turns discharge 5 cc. from either syringe.

For the frogs which we have used adequate perfusion rate was secured by turning the crank through a fraction ($\frac{1}{6}$, $\frac{1}{4}$, $\frac{1}{2}$) of a revolution per second. This was done by hand, the tempo being given by a metronome which was beating seconds.

The frogs used were exclusively *Ranae catesbianae*. Young females supplied the kidneys for perfusion; blood to fill the perfusion apparatus was taken from the aorta of adult females. Coagulation of blood was prevented by the addition of heparin.

Details of the preparations for perfusion are as follows: The frog was pithed: the body cavity opened by a longitudinal incision through skin and muscles a little to the left of the midline, hemorrhage being prevented by cautery. A cannula was inserted into the central stump of the anterior abdominal vein and this was attached to a syringe containing saline.

The large intestine was divided between ligatures, the coeliaco-mesenteric artery tied and cut. The stomach was divided between ligatures and after tying the hepatic portal vein the gastro-intestinal tract and spleen were excised. At intervals during this dissection a little saline was injected into the abdominal vein in order to maintain the circulation. The peritoneum between the right kidney and the body wall was divided and the lateral border of the kidney drawn over the window in the frog board which permitted transillumination. A ligature was placed about the aorta below the origin of the renal arteries and the perfusion cannula inserted into the aorta just above the level of the stump of the coeliaco-mesenteric. The cannula was immediately connected with the perfusion apparatus and a slow stream of well oxygenated Hamburger's solution allowed to flow into the aorta from the perfusion bottle. The aorta below the renals was then tied, and a cannula inserted into the posterior vena cava.

While these preparations were being completed, about 15 cc. of frog's blood was collected from one or two large bull frogs. After adding heparin, thoroughly mixing and filtering through cotton, this blood was divided into two parts: to one was added enough 1:1000 adrenalin solution to make a concentration of approximately 1:500,000: nothing was added to the other portion. One syringe of the perfusion apparatus was filled with the control blood, the other with the adrenalin blood, the syringes placed in their holders and connected with the perfusion cannula.

The frog board was then transferred to the stage of the microscope, to which was attached the photomicrographic camera fitted with a demonstration ocular. The side tube of the perfusion cannula was connected with a membrane manometer and a drop recorder adjusted to record outflow from the post-caval vein.

Emphasis should be laid upon the fact that neither during the dissection nor during the other preparations was the circulation through the kidneys interrupted more than momentarily. As soon as the aortic cannula was inserted perfusion with oxygenated Hamburger's fluid began and this was continued until perfusion with blood was begun during the actual experiment.

Illumination of the kidney was secured by a small arc lamp, the rays of which passed through a solution of copper sulphate and picric acid.

When everything was ready, a suitable field of the kidney tissue having been chosen for observation, and the camera having been approximately focussed, the tube leading from the perfusion bottle of Hamburger's fluid was clamped and the clamp removed from the tube connecting with the syringe filled with control blood. The crank for this syringe was turned at the rate of a fraction of a turn per second. As soon as flow of blood through the capillaries of the visible glomeruli could be seen to be uniform, the camera was accurately focussed upon one glomerulus and pictures were made as rapidly as plates in the camera could be changed. Then, at a signal, perfusion with adrenalin blood from the other syringe was substituted at exactly the same rate, photographs being made as before. Finally, when the adrenalin blood was exhausted control blood was again substituted.

By these procedures we have the means of knowing that blood flow through the kidneys during the control periods and the adrenalin periods is the same: we have a record of perfusion pressure and hence of gross changes in the renal blood vessels; and we have photographic records of one or more glomerular tufts which accurately show changes in size due to the action of the adrenalin.

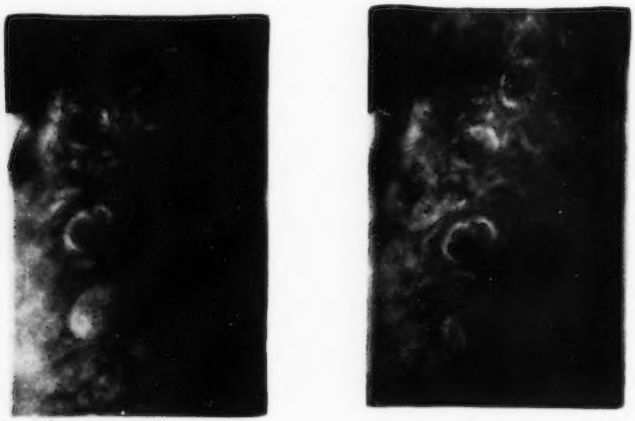
A very slight modification of this technique permitted us to get similar information in experiments in which oxygenated Hamburger's solution was used throughout instead of blood.

Instead of a single perfusion bottle, two were used, their outlets being connected by a Y-tube. To the contents of one a small amount of India ink was added and this was allowed to flow through the kidney for a few moments. Enough of the ink adhered to the glomerular capillaries to make the tuft conspicuously visible. The rest of the experiment was carried out exactly as has been described, the syringes being charged with Hamburger's fluid and Hamburger's fluid + adrenalin respectively.

This arrangement permitted us to observe and record changes in the size of the glomerular tufts which resulted from raising and lowering the perfusion reservoir and hence to compare these with the changes which result from the action of adrenalin.

Two typical experiments, one with blood and one with Hamburger's solution are reported below.

March 24, 1925. Perfusion with whole blood. Small female bull frog pithed. Cannula in anterior abdominal vein by which a fraction of a cc. of Ringer's solution was injected now and then during the preparation. Loose ligatures about aorta and posterior cava. Kidney arranged for observation; active circulation seen in many glomeruli. Tied aorta below kidney: tied coeliaco-mesenteric: eviscerated: inserted aortic cannula: began perfusion with Hamburger: cannula in posterior cava: preparation put on microscope: syringes filled with blood from two other



3-24-25—No. 1 *a* 3-24-25—No. 4 *b*

Fig. 2. Experiment of March 24, 1925. Photomicrographs of glomeruli of a frog's kidney during perfusion at constant rate *a*, with frog's blood; *b*, with frog's blood containing added adrenalin to make 1:500,000. Exposures, 3 to 4 seconds.

frogs. Adrenalin (0.01 cc. 1:1000) added to one to make 1:500,000. Camera ready. Hamburger perfusion discontinued and perfusion with control blood at rate of $\frac{1}{4}$ turn per second begun, and continued for three minutes. Two exposures, second of which was blank. Adrenalin blood substituted and continued for $3\frac{1}{2}$ minutes. Three pictures made. Control blood again. One picture. Perfusion pressure rose noticeably during adrenalin period and sank during second control period. Rate of venous outflow during first control period was 13 drops per minute: during adrenalin period, 12, 12, 12, 12: during second control period 13, 13.

Figure 2 shows pictures 1 (control) and 4 (adrenalin).

Figure 3 is a reproduction of the record of perfusion pressure and venous outflow.

March 28, 1925. Perfusion with oxygenated Hamburger's fluid. A medium sized female bull-frog prepared as in previous experiments. Two perfusion reservoirs, both containing Hamburger's fluid: contents of one tinged with India ink. Allowed enough of the ink-Hamburger flow through to stain the tufts: then changed to Hamburger alone. Photographs were made of a tuft during static perfusion with

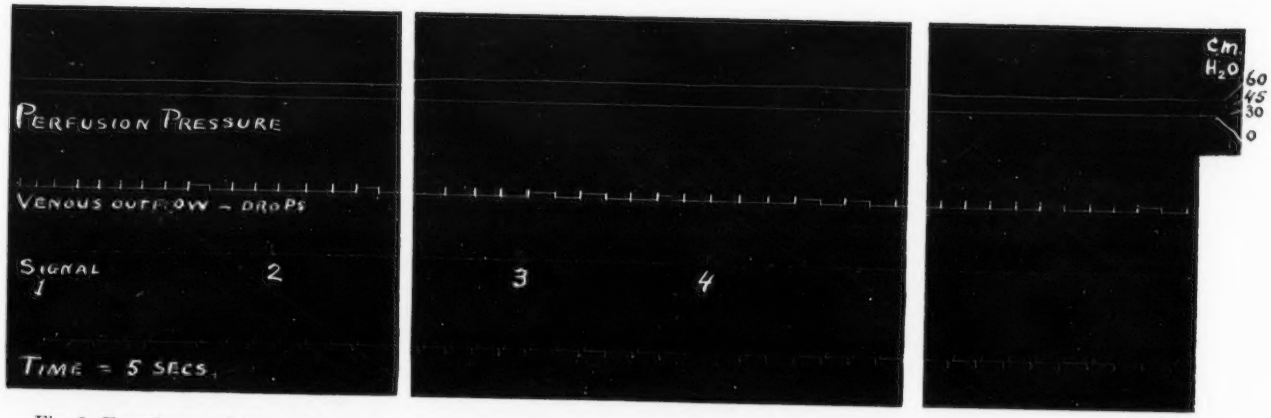


Fig. 3. Experiment of March 24, 1925. Records of perfusion pressure (Hürthle membrane manometer) and drops of blood issuing from the posterior cava during perfusion of the frog's kidney at constant rate: *A* and *C*, with frog's blood; *B*, with frog's blood containing added adrenalin to make 1:500,000; 2¾ minutes elapsed between *A* and *B*, and 1½ minutes between *B* and *C*. The signal line under the drop record shows when the photographs shown in figure 2 were made.

Hamburger's fluid at pressures of 28, 61 and 28 cm. of water (fig. 4, *a*, *b* and *c*). Then syringe perfusion, ½ turn per second; control Hamburger, one picture; adrenalin Hamburger (1:500,000), one picture; control Hamburger, one picture (fig. 5, *a*, *b* and *c*). Did not make record of pressure or venous outflow.

When the results of these experiments are considered in connection with those to which allusion has been made at the beginning of this paper, it may be seen that they fall in place in a line of evidence supporting the view

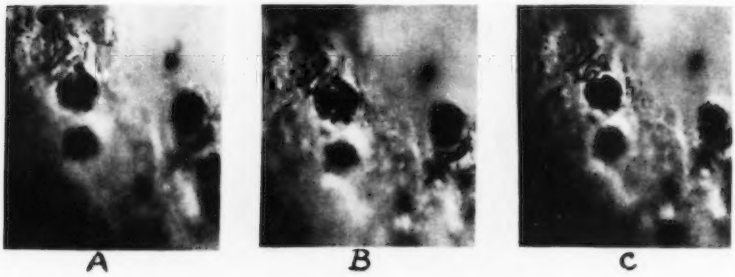


Fig. 4. Experiment of March 28, 1925. Frog's kidney perfused with oxygenated Hamburger's solution, the glomeruli having previously been stained with India ink. *A*, perfusion pressure, 28 cm. *B*, perfusion pressure, 61 cm. *C*, perfusion pressure, 28 cm.

Fig. 5. Experiment of March 28, 1925: same glomeruli as shown in figure 4. Perfusion at constant rate of flow: *A* and *C* with Hamburger's solution; *B* with Hamburger's solution + 1:500,000 adrenalin. One and one-half minutes elapsed between *A* and *B;* 1 minute between *B* and *C*. Exposures, ½ second.

that the calibre of vessels distal to the glomerular tuft is susceptible of alteration by pharmacological agents. In the rabbit's kidney, perfused at constant rate of flow, adrenalin causes constriction of vessels, increase of kidney volume, and diuresis. It is now shown that in a frog's kidney, similarly perfused, constriction of renal vessels under the influence of adrenalin is demonstrable by increase in perfusion pressure, while at the same time the glomerular tufts increase in size. But it is not necessary

to perfuse the kidneys of these animals in order to obtain evidence for this statement. A minute amount of adrenalin in the rabbit has caused lessened blood flow (constriction of vessels), increased kidney volume (dilatation of vessels), and increased urine; and in the frog's kidney with normal circulation it has caused diminished blood flow, and increase in size of the glomerular tufts.

Only one alternative presents itself to the explanation that adrenalin in these dosages caused vasoconstriction distal to the tuft and that is that in the dosages used it possesses the power to cause by direct action dilatation of the glomerular capillaries.

Three observations indicate that this alternative may be dismissed: first, when the frog's kidney is perfused with oxygenated Hamburger's solution at constant pressure (not constant flow) the addition of adrenalin to make a concentration of 1:500,000, or 1:1,000,000 causes flow to diminish and the glomerular tufts to shrink. This experiment is not conclusive because constriction of the afferent vessel together with the effect of existing intracapsular pressure might mask a dilator action on glomerular capillaries. Second: in a kidney perfused via the aorta with Hamburger's fluid at constant pressure, the capsule of Bowman of one glomerulus was so lacerated with a sharp quartz point that positive intracapsular pressure could not develop. Successive measurements of the diameter of the glomerular tuft were made as follows:

	diameter of the glomerular tuft
(a) Perfusion with Hamburger at 34 cm	246 μ
(b) Interruption of (a), intracapillary pressure, 0	193 μ
(c) Perfusion with adrenalin, 1:500,000, at 34 cm	210 μ
(d) Interruption of (c), intracapillary pressure, 0	175 μ
(e) Perfusion with Hamburger at 34 cm	246 μ

These results show that when precautions are taken to reduce both the glomerular intracapillary pressure and the intracapsular extracapillary pressure to zero the size of the tuft is not increased by adrenalin in the concentration tested.

Third: J. M. Hayman (1926), in this laboratory has made measurement of pressure within the glomerular capillaries of the living frog. When adrenalin was introduced into the circulation in dosages of the order of those which Richards and Schmidt found to cause decreased glomerular flow and increased size of tuft, marked increase in pressure within the glomerular capillaries occurred.

These facts indicate clearly we think that the action of adrenalin in the dosages studied does not include a dilator effect upon glomerular capillaries but increases the resistance to outflow of blood from them.

In this paper we have spoken of this increased resistance as being due

to constriction of vessels distal to the glomerular tuft; in earlier papers the belief has been expressed that the vessels concerned were the vasa efferentia. While we have no direct proof that the efferent vessel is constricted to the exclusion of capillaries and veins distal to it, the very fact that the volume of the kidney increases during the constriction which concerns us is evidence that the constriction cannot be occurring in a portion of the vascular bed, having a great total cross sectional area, and in which blood flow is slow. For it is scarcely conceivable that constriction of the tubular capillaries could occur, of such a degree as to retard blood flow through the glomeruli, without producing diminution in the size of the whole organ.

These experiments together with those of Hayman just mentioned, appear to be a final link in the chain of evidence establishing the soundness of the view that intraglomerular pressure and hence also intraglomerular urine formation is susceptible of alteration by influences which affect resistance to blood flow in the efferent vessel to a different degree than in the afferent. Our experiments have largely concerned the influence of adrenalin. But we regard the information obtained from the study of this substance as applicable to the question of mode of nervous control of those vessels upon which adrenalin acts; and the results with pituitrin and with barium in the rabbit's kidney provide evidence that other substances than adrenalin possess the power of affecting the glomerular process in a similar manner.

SUMMARY

The kidneys of frogs have been perfused with frog's blood by means of an apparatus which insures constancy of blood flow. The addition of a small amount of adrenalin to the blood caused the perfusion pressure to rise and the glomerular tufts to swell. The result is interpreted as further evidence that the efferent vessel of the glomerulus is subject to the constricting action of adrenalin.[2]

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[2] All of the experiments described in this paper were finished in March 1925. The saline perfusion experiments have been repeated during April 1926 with completely confirmatory results.

THE ACCESSIBILITY OF THE GLOMERULAR VESSELS TO FLUID PERFUSED THROUGH THE RENAL PORTAL SYSTEM OF THE FROG'S KIDNEY

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Because of its vascular arrangement, perfusion of the frog's kidney has been utilized by a number of experimenters in efforts to obtain evidence bearing upon the function of the renal tubules. The methods used are based upon the belief that a stream of fluid flowing into the renal portal vein does not reach the glomerular capillaries, particularly if these are simultaneously perfused from a system connected with the renal arteries.

Cullis (1906), who first introduced this method, found that venous perfusion with Ringer's solution at 25 to 40 cm. pressure yielded no urine: but that the addition of urea, caffeine, glucose or phlorhizin caused urine flow to be inaugurated. Hence she concluded that these substances stimulate secretion by the tubules. Rowntree and Geraghty (1912) and Rowntree, Fitz and Geraghty (1913) used the same method (35 cm.) in studying the elimination of phenolsulphonephthalein and of lactose, and decided that the tubules take part in the elimination of those substances. But when Bainbridge, Collins and Menzies (1913) showed that fluid, perfused into the renal portal vein at 35 cm., could find entry into the glomerular capillaries, it became certain that the Cullis method did not exclude glomerular elimination of the substances studied. Bainbridge, Menzies and Collins (1914) found, however, that in the great majority of experiments the glomeruli were not involved when the renal portal system was perfused at low pressure (10 to 12 cm.), or even at high pressures if stagnant blood had not been washed out of the glomerular capillaries, and that, under these circumstances, no urine was formed.

On the basis of this work, Atkinson, Clark and Menzies (1921) perfused the arterial system at 24 cm., and the renal portal system at 10 to 12 cm., assuming (with no further proof for the assumption than that which had been supplied by Bainbridge, Menzies and Collins) that under these pressure conditions access of the renal portal fluid to the glomerular capillaries was prevented. This assumption has very generally been made by those who have used the method since.

It is the purpose of this paper to describe experiments which show that

this assumption is unsafe and hence that certain conclusions, drawn from work in which the assumption was made, may not be sound.

Experiments in which the double perfusion method of Bainbridge, Collins and Menzies has been utilized fall into two categories: those in which the chief problem studied has been reabsorption in the renal tubules; and those in which the chief problem was secretion by the tubules.

It is apparent that in certain important experiments in the former class (e.g., Bainbridge, Collins and Menzies; Clark (1922); Yoshida (1924)) no error is introduced even though the fluid perfused through the renal portal vessels mingled in the glomerular capillaries with that infused into the arterial system. Clark, for example, used the same fluid (containing glucose) for perfusion of both systems. He collected a fluid from the ureter containing less glucose than the perfusion fluid. The soundness of his deduction that glucose had been reabsorbed from the tubules is in no wise impaired by the possibility that the fluid entering the renal portal system may have reached the glomeruli; this is also true of the experiments of Schulten (1925) on the concentration of dyes by the perfused kidney. But when Clark perfused a low concentration of glucose through the arterial system and a high concentration through the renal portal system, and concluded that the reabsorptive process ceased when the concentration of glucose in the fluid within the tubular capillaries attained a height of 0.21 per cent, (i.e., the level at which the glucose concentration of the ureteral fluid became identical with that of the arterial perfusion fluid) his estimate would be wrong had the renal portal fluid mingled with the arterial fluid in the glomerular capillaries.

Experiments of the second type are to be found in the work of Atkinson, Collins and Menzies already mentioned and in that of Yoshida. In them, arterial perfusion was conducted at pressures of 24 to 26 cm., renal portal perfusion at 10 to 12 cm. Urea, sodium sulphate, sodium iodide or sodium sulphocyanide was added to the renal portal fluid and when the added substance was identified in the ureteral fluid the conclusion was drawn that it had passed through the walls of the tubules. Obviously the validity of the conclusion would be destroyed if the renal portal perfusion fluid had gained access to the glomerular capillaries.

Evidence is now at hand which indicates the possibility of such intermingling of the two streams within the glomeruli. All who have perfused the renal portal system in the frog have recognized that the resistance encountered is relatively low. Hayman (1926) has measured the pressure in the glomerular capillaries both of living frogs and in frogs' kidneys perfused via the aorta. His two series of experiments agreed in showing that glomerular pressure is on the average about one-half that of the aortic pressure; pressure in the efferent vessel must be less than this. These facts indicate that when the head of pressure in the aortic system is only

double that in the renal portal system, access of renal portal fluid to some glomeruli may be expected. It may also be conceived that not all of the afferent arteries and arterioles in the frog's kidney are patent to an arterial perfusion at 24 cm. pressure: and there is no evidence upon which to base a denial of the possibility that efferent pathways may be open to glomeruli whose afferent pathways are closed.

Cushny (1926), in his most recent discussion of the evidence which has been derived from perfusion of the frog's kidney, recognized clearly the possibilities of error to which we are alluding: but directly sustaining evidence was not available.

METHODS. *R. pipiens* and *R. catesbiana* were the species of frogs used. The brain was destroyed by pithing before beginning the dissection. The usual perfusion technique was employed. For perfusing the renal portal system, a cannula was inserted into the anterior abdominal vein, the thighs being ligated: for the arterial perfusions, a cannula was inserted into the central stump of the abdominal aorta below the origin of the renal arteries, the aorta and coeliaco-mesenteric artery having been tied above. The perfusion fluids left the kidney either by an opening made into the posterior vena cava or by a cannula inserted into this. When large frogs were used, urine was collected by cannulae in the ureters; when small frogs were used, a cannula was tied into the cloaca.

The perfusion fluid employed was Hamburger's solution or Ringer's solution. In making the solutions, glass distilled water and Kahlbaum's guaranteed chemicals were employed. All solutions were thoroughly oxygenated.

I. URINE FORMATION DURING VENOUS PERFUSION ALONE AT HIGH PRESSURES. In a number of experiments some of the conditions studied by Cullis and by Bainbridge, Menzies and Collins were imitated. These experiments will be summarized only.

1. *Renal portal perfusion with saline.* In one experiment with Hamburger's fluid in which the venous perfusion pressure was 15 cm. of water, no fluid issued from the kidney during an experiment lasting five hours.

In five experiments in which the venous perfusion pressure was 35 to 40 cm., no fluid left the kidney in experiments lasting from one to two hours.

These results are like those of Cullis and differ from those of Bainbridge, Menzies and Collins (seven out of twelve experiments). There is reason for thinking that discrepancies such as these may be attributed to differences in actual pressures within the vessels perfused: for in renal portal perfusions of this type we have found that urine may be caused to flow by simple obstruction to venous outflow from the kidneys.

2. *Renal portal perfusion with 3 per cent urea.* Cullis found that the addition of urea to the venous perfusion fluid invariably resulted in secretion.

In two of our experiments in which the perfusion fluid contained 3 per cent urea and the perfusion pressure was 15 cm., no urine appeared in experiments lasting from one-half to one hour. In three experiments in which perfusion pressure was 20 to 25 cm. minute amounts of urine appeared after 35 to 55 minutes. In twenty-two experiments in which the perfusion pressure was 30 to 40 cm., urine always appeared in less than 40 minutes, usually in less than 15 minutes, and continued throughout the duration of the experiments. Although we usually perfused the aorta for ten minutes preliminary to the venous perfusion, in three experiments this was not done; but there was no significant difference in the subsequent urine flow. In the majority of these the rate of urine formation by each kidney was between 0.2 and 0.4 cc. per hour.

Furthermore, in several experiments in which no urine was being formed, obstruction of the renal veins resulted in prompt inauguration of urine flow.

These results corroborate the evidence of Bainbridge that urine formed during renal portal perfusion varies with perfusion pressure and support his view of its glomerular origin.

In three of our experiments, after urine flow had been established by venous perfusion with urea at high pressure, reduction of the pressure did not then cause cessation of urine flow. In three experiments, after urine flow had been established by renal portal perfusion with urea at high pressure, substitution of saline containing no urea did not cause urine flow to cease. This suggests that dilatation of the efferent vessels is caused by the high pressure and the urea content of the renal portal fluid, and that this is not abolished by lowering the pressure or removing the urea.

3. *Outflow of fluid from the aorta during renal portal perfusion.* In thirteen renal portal perfusions at 35 to 40 cm. with Hamburger's solution containing urea, a cannula was inserted into the aorta to permit the collection of fluid which might flow from the venous system through the glomeruli into the renal arteries. In eleven experiments such reverse flow occurred, and in ten of these the time relations between occurrence of the reverse flow and the inauguration of urine flow was such as to indicate causal connection between the two. The volume of aortic reflux was roughly proportional to the rate of urine formation.

In the two previous sections we have stated that obstruction of the renal vein caused inauguration of urine flow in several instances of renal portal perfusion with or without urea. In one experiment it was found that conspicuous increase in the rate of aortic reflux occurred immediately upon obstructing venous outflow.

These results are similar to those of Bainbridge, Menzies and Collins.

4. *Staining of glomerular capillaries by a dye added to the renal portal perfusion fluid.* In their most conclusive experiments, Bainbridge,

Menzies and Collins showed that Prussian blue, added to the renal portal perfusion fluid, could be detected in sections of the glomeruli of kidneys which had formed urine during the perfusion; not in those of kidneys which had formed no urine. This result was confirmed in five of our experiments in which Janus Green B was similarly perfused at high pressure. While this dye is not as advantageous for staining glomerular vessels by venous perfusion as by aortic perfusion because of the more intense stain taken by the tubules, the results were unmistakable in showing that the renal portal fluid had reached the glomeruli.

5. *Influence of colloid in the perfusion fluid on the production of urine during renal portal perfusion.* In five experiments urine flow was established by perfusing the renal portal system with Hamburger's fluid, to which was added urea (3 per cent), at pressures of 30 to 40 cm. In two instances the addition of acacia to the perfusing fluid in amount sufficient to make a concentration of 3 per cent caused complete cessation of urine flow. In two other instances urine flow from one kidney ceased, while that from the other was markedly decreased: and in the fifth instance, the urine flow was diminished to about one-tenth of its previous rate.

In all of these experiments the addition of the colloid to the perfusion fluid decreased the rate of flow through the renal portal system; but it did not fall below the rates at which, in other experiments without colloid, urine had been formed in considerable amounts.

6. *Partial occlusion of the glomerular vessels by intra-arterial injection of starch.* With the thought that it might be possible to prevent access of renal portal fluid to the glomerular capillaries by filling these with starch granules, a suspension of starch was prepared and in six experiments injected into the renal arteries by way of the aorta at a pressure of 30 cm. The injection was continued until permanent obstacle to the continued flow of fluid into the aorta was produced. The starch injection did not influence the rate of flow of the simultaneous renal portal perfusion. The effect of these injections upon the amount of urine resulting from renal portal perfusion with urea at 30 to 40 cm. was not conclusive. The amounts of urine formed were smaller and the onset of urine formation was slower than was usual in such experiments in which no starch had been injected; but when paraffin sections were made of the starch-injected kidneys it was found that the starch had failed to lodge in many glomerular capillaries, and was most abundant in the renal arteries and arterioles.

In this connection it is interesting to note that if the kidney be perfused with Hamburger's solution via the aorta, resulting flow of fluid from the ureters is not stopped or materially diminished by ligating all venous outlets of the kidney—i.e., circulation of fluid through the glomerular capillaries is not a necessary requisite for the formation of urine from a colloid-free perfusion fluid.

7. *The passage of glucose and of dyes into the urine formed during renal portal perfusion.* In two experiments urine flow was initiated by perfusion of the renal portal system with Hamburger's solution containing urea at 30 to 40 cm. pressure. The addition of indigocarmine, toluidin blue, or glucose to the perfusion fluid was followed by the appearance of the added substance in the ureteral fluid. None of these substances when added to the renal portal fluid in the absence of urea produced elimination of urine.

8. *Influence of mercuric chloride upon urine formation during renal portal perfusion.* In two experiments renal portal perfusion with Hamburger's fluid containing 3 per cent urea at 40 cm. was interrupted for 10 to 20 minutes while 1:10,000 mercuric chloride solution was allowed to flow through the system at a pressure of 12 cm. The conditions of the original perfusion were then restored. Urine flow was resumed at its original rate. The sodium chloride content of the urine before mercuric chloride was less than that of the perfusion fluid; after mercuric chloride it was approximately the same. In another experiment ethyl urethane (0.1 per cent) was added to the renal portal fluid. Urine flow continued without change in rate.

The chief contention advanced by Bainbridge years ago was that when the renal portal system is perfused at high pressure, as in the experiments of Cullis, access to the glomerular vessels is possible and the urine which results is glomerular in origin. From a review of the experiments which we have summarized, it will be seen that this contention is upheld by a number of confirmatory observations: the intimate connection between perfusion pressure and urine formation; the passage of the perfusion fluid through the glomeruli into the aorta; staining of the glomeruli by dye introduced with the perfusion fluid; the influence of the presence of colloids in the perfusion fluid upon urine flow; failure to affect urine flow by addition of mercuric chloride to the perfusion fluid. In no case has participation of tubule function been identified, and in every case the result has been that which was anticipated on the assumption that the ureteral fluid was eliminated in the glomeruli.

II. Perfusion of the renal portal system at low pressure. In the Prussian blue injection experiments of Bainbridge, Collins and Menzies no dye was found within the glomeruli when it was perfused through the renal portal system at a pressure of 10 to 14 cm. Our observations do not agree with this.

1. In four experiments cannulae were inserted for perfusion of both the renal portal and the arterial systems. After Ringer's fluid had been allowed to flow through both for 5 to 30 minutes, the aortic perfusion was discontinued. Ferric ammonium citrate was added to the renal portal perfusion to make a concentration of 1:1000, and urea to a concentration of 0.02 per cent, perfusion pressure being 12 cm. After this perfusion had

continued for from 5 to 30 minutes an acidified solution of potassium ferrocyanide was injected into the arterial system by means of a syringe. The kidneys were at once excised, placed in 10 per cent formalin, and frozen sections cut. In all four experiments the great majority of glomerular tufts were stained.

2. In seven experiments the renal portal vein was perfused with Ringer's solution containing a dilute suspension of India ink[1] for 30 minutes at 10 to 12 cm. pressure. In five of these the arterial system had been washed out with Ringer's solution for 5 to 10 minutes previously; in the other two the aorta had simply been ligated before beginning the renal portal perfusion. No urine was formed in any of the seven experiments. Frozen sections were made and the glomeruli examined. One hundred glomeruli were counted. In the five experiments in which the aorta had been perfused before introducing the ink into the renal portal perfusion, from 20 to 67 per cent of the glomeruli contained considerable deposits of ink.

In one of the two in which the aorta had not been perfused all of the glomeruli counted contained ink; in the other, 20 per cent contained moderate amounts.

3. Perfusion of the renal portal system alone with oxygenated Ringer's or Hamburger's fluid at 10 to 12 cm. does not give rise to urine formation. If, however, mercuric chloride be added to the solution to make a concentration of 1:10,000, fluid soon begins to appear in the ureteral cannulae and continues to flow during the rest of the experiment. Six experiments of this type have been made, in five of which the aorta was tied above and below the origin of the renal arteries, no aortic cannula having been inserted. In every instance ureteral flow began within 30 minutes from the beginning of the mercury perfusion; in one instance it continued for six hours. The ureteral fluid was collected and analyzed in five instances. Its chloride concentration was found to be approximately the same as that of the perfusion fluid.

The glomerular origin of this ureteral fluid is indicated by the following observations:

a. Injection of starch granules into the renal arteries previous to, or early in the course of, the mercuric chloride perfusion was found to prevent or to check the ureteral flow in the three experiments in which it was tried.

b. In one experiment, 20 minutes after the beginning of perfusion of the renal portal vessels with mercuric chloride, 1:10,000, at 12 cm. pressure, ferric ammonium citrate was added to the perfusion fluid. Fifteen minutes later, acidified potassium ferrocyanide was injected through the renal

[1] Weber's (Philadelphia) India ink, stated to have been made by Higgins, was used. The dilution in this and subsequent experiments was five drops of ink to 100 cc. of fluid.

arteries. In sections, Prussian blue was seen in a large number of glomeruli.

c. In one experiment (the only one in which the test was made) reflux of the renal portal fluid into the aorta was observed to follow the addition of $HgCl_2$ to the perfusion fluid.

The experiments summarized in this section show clearly that fluid perfused through the renal portal system at 12 cm. pressure finds access to the glomeruli by way of the efferent vessels—a result which differs from those obtained by Bainbridge, Collins and Menzies and previously by Beddard (1902). The following experiments throw some light on this discrepancy. A renal portal perfusion was established at 12 cm. pressure with oxygenated Ringer's solution to which a little India ink had been added. After 30 minutes a saturated solution of soluble Prussian blue (Grübler) in water was substituted, and the pressure raised to 28 to 30 cm. After 4 minutes' perfusion with this fluid the kidney was excised, put into alcohol, then into 10 per cent formalin, and frozen sections cut. Ink was found in 48 out of 100 glomeruli and blue in only 2. In another experiment, like this except that the Prussian blue was perfused for 8 minutes, 94 out of 100 glomeruli contained ink and only 26 blue. Bainbridge called attention to the viscidity of the Prussian blue solution which he used. Our experiments show that brief perfusion with Prussian blue even at high pressure will not reveal with certainty the distribution of saline previously perfused for a long time at low pressure.

To the summary of these experiments in which renal portal perfusion pressures of the order of 12 cm. have been shown to enable access of the perfusion fluid to the glomeruli, may be added a brief allusion to two experiments which indicate that under certain circumstances far lower pressures may be similarly effective. In one, the aorta was ligated above and below the renal arteries and the renal portal system perfused with oxygenated Ringer's solution at 14 cm. pressure for ten minutes; the renal portal perfusion was then discontinued and the cannula in the posterior vena cava filled with Ringer's solution containing a little India ink; 0.2 cc. of this fluid was allowed to flow into the kidney vessels at a pressure of 3.5 cm. during a period of eight minutes. Ten minutes later the kidneys were excised and sections cut. Some 15 per cent of the glomeruli examined contained traces of ink.

In another experiment, made by Doctor Barnwell and one of us, the aorta was similarly tied, and the right renal portal vein perfused for 23 minutes at 12 cm. pressure. On clamping the tube from the renal portal perfusion bottle a little blood—obviously from the veins of the left kidney—could be seen to collect in the cannula in the posterior vena cava. This cannula was held upright and, watching the glomeruli of the right

kidney under the microscope, blood cells could be seen to enter the glomerular capillaries and in some cases to pass through into the afferent vessels. The pressure of the blood in this instance was not more than 2 cm.

Hence, while we conclude that 12 cm. pressure in the renal portal system is effective in causing entrance into the glomerular vessels, we have reason to believe that pressures far lower than this may be similarly effective.

In another respect our results differ from those of Bainbridge, Menzies and Collins. They decided that when the arteries to the kidney were ligated so that blood remained occluded in the glomerular vessels, fluid subsequently introduced via the renal portal vein even at high pressures did not enter the glomeruli. In the three experiments alluded to in our first section, perfusion pressures 30 to 40 cm., and in the two experiments referred to in this section (2), perfusion pressures 10 to 12 cm., ink was found to have entered the glomeruli. Stagnant blood does not quickly clot in the glomerular vessels, and it seems probable that Bainbridge misinterpreted his findings in this connection.

III. Simultaneous perfusion of the renal portal system and the arterial system. Bainbridge, Collins and Menzies were the first to adopt the plan of simultaneously perfusing the renal arteries at pressures of 20 to 25 cm., and the renal portal system at 10 to 12 cm., pressures which they conceived to approximate those of the intact frog. At these pressures they perfused dilute mercuric chloride through the renal portal system while Ringer's solution flowed through the arterial system: they identified the distribution of mercury by fixation as sulphide. They state that it is difficult to demonstrate the absence of mercury from the glomeruli, but in most cases, and in so far as they could judge, they were free. In some cases it appeared to be definitely present in the glomeruli "probably because of diffusion." As the surest method of confining the poison to the tubules they recommend very brief perfusion of the renal portal vein at "very low" pressure. In connection with their failure to find in the glomeruli Berlin blue added to the renal portal fluid, they state that "Berlin blue is distinctly more viscid than saline." For these reasons their account does not indicate that they were completely convinced that fluid introduced at 10 to 12 cm. pressure into the renal portal vein for a prolonged period could not gain access to the glomeruli, despite simultaneous arterial perfusion at 24 cm. pressure: nor do we find that this doubt has been laid by those who have utilized the method since.

For the experiments of Bainbridge, Collins and Menzies the possibility of access of traces of the renal portal fluid to the glomeruli does not materially influence the validity of the conclusions which they drew; for these were chiefly concerned with the problem of reabsorption from the tubules: but it does influence materially the acceptability of conclusions which

others have drawn from experiments similarly conducted in which the problem has chiefly concerned secretion by the tubules.

Our experiments give clear evidence that renal portal fluid at 10 to 12 cm. pressure may and commonly does reach the glomeruli even though arterial perfusion at 24 cm. is simultaneously conducted.

1. In ten experiments in which the arterial and renal portal systems have been simultaneously perfused with Hamburger's fluid at 25 and 12 cm. pressure respectively, we have added ferric ammonium citrate to the renal portal fluid to make a concentration of 1:1000. After from 5 to 45 (usually 10) minutes, the venous perfusion was stopped and the aortic cannula connected with a syringe containing 1:1000 potassium ferrocyanide made faintly acid with HCl. Injection of this fluid into the renal arteries caused the kidneys to become diffusely blue, and the fluids which issued from the ureter and from the posterior vena cava became blue. In some instances the visible renal arteries appeared blue. The kidneys were promptly placed in 10 per cent formalin and frozen sections cut. A number of glomeruli were examined—usually one hundred—from different parts of the kidney.

In five experiments in which the renal portal fluid contained 0.02 to 0.04 per cent urea, 76 to 100 per cent of the glomeruli examined contained large amounts of Prussian blue.

In five experiments in which the renal portal fluid was identical with the arterial (no urea) the number of glomeruli heavily stained with Prussian blue was from 20 to 51 per cent: smaller amounts were contained in from 63 to 17 per cent: and none at all could be seen in 5 to 34 per cent.

In two additional experiments, the perfusion pressures were 40 and 10 cm. respectively: no urea was added to the renal portal fluid. Prussian blue was found in considerable quantities in 9 to 36 per cent of the glomeruli; small amounts in 44 and 53 per cent; and none at all in 47 and 11 per cent.

2. Fourteen experiments were made in which the arterial and renal portal systems were simultaneously perfused with Ringer's solution at 24 and 12 cm. respectively, India ink being added to the renal portal fluid. In eight of these the renal portal fluid contained 0.02 to 0.04 per cent urea. After thirty minutes, the perfusions were discontinued, the renal portal first. The kidneys were promptly placed in 10 per cent formalin solution and frozen sections cut. Distribution of the ink within the kidneys was not wholly uniform, the lower poles being most deeply stained. Sections were usually made from the lower half. Glomeruli were examined and counted as in the experiments described in the previous section.

In the eight experiments in which the renal portal fluid contained urea, 82 to 100 per cent of the glomeruli examined contained ink in various amounts. (See fig. 1.) There was no ink in 0 to 11 per cent. We omit

from these figures record of glomeruli in which questionable traces may have been present.

In five of the six experiments in which no urea was contained in the renal portal fluid, considerable amounts of ink were found in 45 to 88 per cent of the glomeruli examined; none in 55 to 12 per cent. In the sixth experiment of this group ink was unmistakably present in only 21 per cent.

In three additional experiments the perfusion pressures were 40 and 10 cm. respectively. No urea was added to the renal portal fluid. From 22 to 51 per cent of the glomeruli examined in these kidneys showed unmistakable deposits of ink, and 55 to 36 per cent showed none.

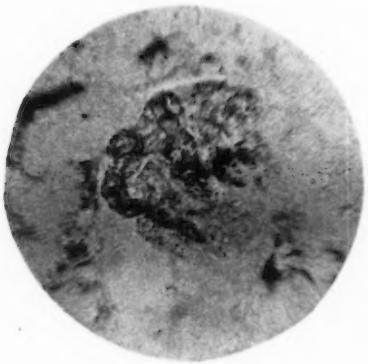

Fig. 1. Photomicrograph of a glomerulus showing deposition of India ink within its capillaries. The renal arteries had been perfused with Ringer's solution at 24 cm. and the renal portal vessels with Ringer's solution containing 0.04 per cent urea at 12 cm. for 30 minutes. India ink was added to the renal portal fluid only. No counter stain. Magnification, 110.

3. In two experiments arterial perfusion with Hamburger's fluid was conducted at 24 cm. pressure, and renal portal perfusion at 12 cm. with the same fluid to which urea had been added to make 0.02 per cent. After 30 minutes, $HgCl_2$ was added to the renal portal fluid to make 1:10,000. This was allowed to flow for 20 to 25 minutes, after which the original Hamburger-urea was substituted. Urine samples collected before the bichloride perfusion contained urea; their chloride concentration was less than that of the perfusing fluid. Urea continued to be present in the ureteral fluid after the bichloride perfusion, in concentration comparable to that in the former samples in so far as could be judged by the

hypobromite test; chloride concentration, however, became equal to that of the perfusing fluid.

In these experiments urea was supplied to the kidneys only by way of the renal portal vessels. Tubular activity was believed to have been eliminated by the action of mercuric chloride. The continued elimination of urea therefore indicates that the renal portal fluid had access to the glomeruli.

4. A more graphic demonstration of the access of fluid to glomeruli via the efferent vessel was obtained in four experiments made as follows: Ringer's solution was perfused through the renal arteries at 30 cm. pressure and simultaneously Ringer's solution containing 0.02 per cent urea through the renal portal system at 12 cm. After 10 minutes a very fine suspension of acid carmine was added to the arterial reservoir and a little India ink to the venous. The double perfusion was continued for 15 to 25 minutes thereafter. Frozen sections of these kidneys showed only carmine in some glomeruli (13 to 34 per cent); only ink in others (12 to 36 per cent); and both carmine and ink in still others (35 to 66 per cent).

The relatively large proportion of glomeruli in which both substances were present confirms the results of the other experiments described in this section. Particular interest attaches to the finding of ink alone in a certain number of glomeruli; it indicates easier access of fluid to these glomeruli by way of the efferent than by way of the afferent vessels; and for the first time, in so far as we are aware, it provides direct evidence that, under accepted conditions of double perfusion of the frog's kidney, a considerable fraction of the total number of glomeruli in the kidney may be reached only by the fluid introduced through the renal portal vein.

The results outlined in this section yield convincing evidence that fluid perfused through the renal portal vessels enters many glomerular capillaries despite simultaneous perfusion of the arterial system at pressures from two to four times as great as that which obtains in the renal portal. They also show that the glomerular distribution of the renal portal fluid under these conditions is increased by the addition of urea in small amounts to the renal portal fluid.

SUMMARY

The method of perfusion of the frog's kidney as introduced by Cullis and modified by Bainbridge, Collins and Menzies has been used in the experiments summarized in this paper in an effort to test the validity of this mode of approach to the problem of secretion by the renal tubule.

In confirmation of Cullis it was found that when the renal portal system only was perfused at high pressures (30 to 40 cm.) with Ringer's or Hamburger's fluid containing urea, urine is formed. This result is regarded,

however, as showing elimination through the glomeruli rather than through the epithelium of the tubules: 1, because of the proportionality observed between the perfusion pressure and the rate of urine formation; 2, because of the passage of the venous perfusion fluid through the glomeruli into the aorta; 3, because of the identification within the glomerular structures of a soluble dye added to the renal portal perfusion fluid; 4, because the addition of colloid to the perfusion fluid checks the urine flow; and 5, because the same result was obtained in kidneys which had been subjected to the damaging action of mercuric chloride for the purpose of eliminating function of the tubular epithelium. Most of these items are confirmatory of the results of Bainbridge upon which was based his critique of the conclusions reached by Cullis. We differ from him, however, in finding that ligation of the aorta before the beginning of the renal portal perfusion does not prevent the formation of urine.

When pressures of 10 to 12 cm. in the renal portal perfusions were employed, urine was not formed except in experiments in which mercuric chloride (1:10,000) was added to the perfusion fluid. In all cases, however, the renal portal fluid reached a large proportion of the glomeruli; this was demonstrated by the identification in them of an iron salt or of India ink previously added to the venous perfusion fluid. In this result we differ from Bainbridge. We attribute this difference to his selection of measures not calculated to show with certainty the glomerular distribution of the renal portal fluid. He was probably not aware that glomerular urine may be formed under circumstances which do not entail elimination of urine from the kidney.

By similar methods it was found that perfusion of the arterial system at high pressures (24 to 40 cm.) during renal portal perfusion at low pressures (10 to 12 cm.) did not prevent access of the renal portal fluid to the glomeruli. In many there was evidence of mixture of the two fluids; in some, evidence that the arterial fluid alone had entered; and in others evidence that only the renal portal fluid had entered. We explain this result by reference to Hayman's recent determinations of pressure within the glomerular capillaries, and by direct evidence pointing to different degrees of patency of afferent and efferent vessels.

To an unexpected degree, the results indicate that fluid introduced by the renal portal vein finds easy access to the glomerular capillaries: and we conclude that experiments in which the Bainbridge method of double perfusion of the frog's kidney has been used do not provide the basis for a decision that substances introduced into the renal portal vein enter the lumen of the tubules without having passed through the glomerular membranes.

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STUDIES ON THE CONDITIONS OF ACTIVITY IN ENDOCRINE GLANDS

XX.[1] The Influence of Motion and Emotion on Medulliadrenal Secretion

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The great importance of emotional excitement in the determination of conduct, in the mobilizing of bodily forces, and in the disturbance of essential functions gives peculiar significance to a search for facts which might help to disclose its physiological nature. When the search is directed towards elucidating the rôle of an agent, such as adrenin is, which itself can extensively and deeply affect organic processes, it holds a special interest. There is, consequently, good reason for the vigor of the discussion which has continued throughout the past decade relative to the existence of an increased secretion from the adrenal medulla, particularly during intense emotional stress.

Review of literature. Fifteen years have passed since Cannon and de la Paz (1911) announced an emotional stimulation of medulliadrenal secretion. They noted that the beating intestinal strip was inhibited by blood taken from an excited animal, whereas it was not inhibited by blood taken from the same region of the same animal when calm. The next year (1912) Elliott reported that when he compared the adrenin content of the innervated with that of the denervated adrenal gland of cats excited by "β-tetra" or morphine, he found the content in the innervated gland much less than in the other. Furthermore, he observed that paradoxical dilatation of the pupil, which had been disconnected from the superior cervical ganglion, occurred even in slight anger when the adrenal relations were normal, but that no trace of the reaction remained in anger aroused after

[1] The paper entitled "A Lasting Preparation of the Denervated Heart," which was numbered XVII in this series, should have been numbered XIX; number XVII was published in This Journal, 1925, lxxiv, 291.

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the glands had been excised. These results were regarded as bringing prompt support to the view that in fear and rage medulliadrenal secretion is increased.

Elliott's observations on the iris sensitized by removal of the superior cervical ganglion were confirmed by Kellaway (1919). The pupil on the operated side under bright illumination is usually smaller than its fellow. Kellaway laid special stress on the persistently *large* size of that pupil after a test and thereupon its much slower return to a small size, as compared with the normal. With splanchnic nerves intact and adrenal glands present the paradoxical dilatation occurred under excitement, but after the nerves were cut the reaction did not appear. Although the "paradox" was not marked, it was described as "definite."

In 1919 Cannon published electrical records of the effects of excitement on the rate of the heart, after vagus section and stellate removal, when the adrenals were active and again after they had been removed. A change from a calm state to excitement increased the rate 38 beats per minute under the former circumstances and only 4 beats after adrenalectomy. Hartman, McCordock and Loder (1923), using as an auto-indicator the completely denervated iris, reported very little if any increase in the output of adrenin in their cats when in the presence of a barking dog. They testified, however, that this was what might have been expected in animals which did not become excited, explained that there was indifference to the dog while the observers were near, and suggested that further tests should be made on cats unaccustomed to the laboratory.

Besides this more or less direct evidence regarding discharge of adrenin in strong emotional states there have been observations possessing inferential value. Cannon and Mendenhall (1914) reported that stimulation of the splanchnic nerves shortens the coagulation time of blood, just as injected adrenalin does, but that this effect fails if the adrenal glands have been removed; and, furthermore, they found that excitement induces faster clotting, but only so long as the splanchnic nerves are intact. It would appear, therefore, that excitement produced the phenomenon *via* increased medulliadrenal secretion. Also Lamson (1915) noted that fear or rage, as well as injection of adrenalin, evokes in cats polycythemia, but that emotional disturbance after removal of the adrenal glands causes no increase in the red-corpuscle count. Finally, Cannon and Britton (1925) described a method of destroying the lateral cerebral cortex and leaving the basal ganglia, thus producing a pseudaffective preparation which manifests in an acute experiment the physiological phenomena of great emotional excitement. In the pseudaffective state there is evidence of much augmented adrenal secretion, for with the adrenals intact the denervated heart beats very rapidly (averaging 252 beats per minute during activity), and after adrenal removal much more slowly (averaging 156

beats per minute). All these observations taken together are consonant with the view that in periods of tense excitement the adrenal medulla, like other viscera innervated by sympathetic fibers, is involved in the emotional response.

The foregoing review of the evidence favorable to emotional stimulation of an increased adrenin output shows that, besides being indicated by faster coagulation, polycythemia, and a more rapid pulse in the pseudaffective state, it is based on 1, the presence of changes in the adrenin content of the glands; 2, pupillary reactions, and 3, the effects of blood on the beating intestinal strip.

Opposed to this evidence are the experiments and conclusions of Stewart and Rogoff. In 1916 they confirmed Elliott's observations on the effects of morphine and "β-tetra" on the adrenin content in cats; but they drew different inferences. They first discounted the effects of morphine as being due to "fright," because they found that morphine lessens the adrenin content equally well in dogs which are not put into a state of "fright" by morphine, and also because they could not demonstrate any reduction of the adrenin store in cats excited for some time by barking dogs. Furthermore, they failed to confirm the paradoxical reaction,—in four cats observed by them the "denervated" iris and the normally innervated iris both enlarged, "instantaneously," in sudden fright after inactivation of the adrenals. Again (1917), they failed to confirm Cannon and de la Paz; they repeated in three animals the procedure employed, but the blood which they took from the excited animal, they stated, caused no inhibition of the rhythmically contracting intestine. In 1923, without further experimenting, they characterized the "exploits of the barking dog in frightening epinephrin out of the adrenals of a cat" as a "myth."

With regard to the action of the two drugs, morphine and "β-tetra," it may be admitted at once that they complicate the reaction. In cats and dogs they may directly stimulate adrenal secretion, and also they may prevent a restorative process which, in spite of discharge of adrenin, would maintain the supply (cf. Stewart and Rogoff, 1917). Evidence indicates that such a restorative process is normally operating. For example, Tscheboksaroff (1910) and Elliott (1912) observed that splanchnic stimulation, though it is known to produce abundant discharge, leaves a larger adrenin store in the stimulated than in the unstimulated gland. Further, Stewart and Rogoff (1916) reported that during prolonged, interrupted stimulation an amount of adrenin must have been formed in the gland equal to the initial load; and they also found (1919) that after the greatly increased output evoked by strychnine there is no difference in the residual adrenin content of the innervated and the denervated gland. As Stewart (1922) states, assays of the store of adrenin in the gland cannot be used directly as evidence regarding the discharge—"The store represents the

balance between epinephrin formed and epinephrin liberated, and a diminution in the store may be due either to diminished up-building or to increased discharge." From Stewart's own reasoning, therefore, the absence of any reduction of the store in the excited cats studied by him and Rogoff cannot be taken as proof that during the excitement no discharge occurred. The histological observations made on the adrenal medulla of frightened guinea pigs by Buscaino (1920) were negative and likewise not decisive. And the same comments may properly be made regarding the experiments reported by Uno (1922) who compared extracts of the adrenals taken from rats which had been fighting each other for some hours, with extracts taken from resting rats of the same litter. The results were negative. It should be clear that, confronted by positive evidence for adrenin secretion during emotional stress, all this testimony would have no pertinence whatever.

With regard to the pupillary reactions the difference between the observers is sharp. Elliott and Kellaway both state explicitly that the paradoxical pupillary response is present in excitement if the adrenal relations are normal, and that it disappears after adrenalectomy. Stewart and Rogoff saw no difference in the reaction of the "denervated eye" whether the adrenals were intact or inactivated. The main points which they make are that in the four cats with a "denervated eye" and with adrenal secretion eliminated dilatation of the pupil occurred "instantaneously," after an interval much too short for a humoral effect, and that it occurred in the "denervated eye" as well as in the normal eye. As Kellaway pointed out, it is not the rate of dilatation which is watched in the paradox, but the degree of dilatation and the speed of recovery. In the true response the pupil on the operated side becomes regularly larger than the normal and recovers more slowly. And as one of us (Cannon, 1915, p. 35) has remarked before, with regard to this testimony of Stewart and Rogoff, the eye still innervated by the third cranial nerve can hardly be regarded as "denervated;" the "instantaneous" dilatation of the "denervated" iris in rage, therefore, is readily explicable as due to central inhibition of the tonically active constrictor impulses. On the one hand, then, are the observations of Elliott and Kellaway, on excited animals before and after adrenalectomy, which led them to report the failure of the characteristic paradoxical pupillary response in the absence of medulliadrenal secretion; and on the other hand are the observations of Stewart and Rogoff that even after adrenal inactivation there occurs in excited animals a quick widening of the pupil—a phenomenon, however, properly accounted for by inhibition of constrictor tone. Doubtless this nervous factor was present as part of the paradoxical response, and to that extent the test is not above criticism. In the instantaneous change noted by Stewart and Rogoff, however, there is nothing which confutes the conclusions of Elliott and Kellaway that the paradoxical pupillary response signals increased adrenal secretion at times of emotional stress.

Stewart and Rogoff's failure in three attempts to obtain the results obtained by Cannon and de la Paz is not surprising. As already stated (Cannon, 1919), the method is difficult and exacting, and in the hands of the original investigators it was not successful until after considerable experience. The fact is manifest, however, that in their hands it did yield evidence of emotional stimulation of medulliadrenal secretion, and that in the hands of Stewart and Rogoff it did not. Even if Stewart and Rogoff had succeeded, the results, in their opinion, would not have been significant, for they (1920, p. 551) have argued that splanchnic vasoconstriction would cause "slowing of the blood flow in the inferior cava" and therefore greater concentration of the adrenin content without augmented secretion. This argument, it should be noted, is not accompanied by the slightest hint of evidence that the cava flow is in fact retarded. Indeed, Edwards' (1914) demonstration that splanchnic stimulation *increases* the blood flow in the external jugular and in the femoral veins, by 12.5 and 15 per cent respectively, thereby compensating for the check in the portal circuit, they dismiss with the remark that they are "not concerned" with it. Clearly, the faster flow in the inferior cava (associated with the faster flow in the femoral veins) would be unfavorable, rather than favorable, to the results obtained by Cannon and de la Paz. Stewart and Rogoff's further arguments, that those results might be due to the influence of respiration on the cava flow, or to massage of the adrenal medulla by the catheter in the cava, or to a happy placing of the eye of the catheter near an opening of the adrenal veins despite a carefully measured position above them, are so extremely hypothetical, so lacking in any factual support, that they need not be further considered.

THE METHOD. Because of the importance of bodily reactions in strong emotional states, and because of the difficulties which have been encountered in the use of the methods described above, it seemed worth while to attack the problem by way of another procedure. We have made use of the lasting preparation of the denervated heart, in animals operated upon by surgical methods and living normally, as described by Cannon, Lewis and Britton (1926). The results obtained by use of the denervated heart have been questioned by Stewart and Rogoff (1920). With reference to reflex stimulation or asphyxial stimulation, or both, these results, however, have recently been confirmed by Tournade and Chabrol (1925) in Algiers, working on a crossed circulation; by Houssay and Molinelli (1924, 1926) in Buenos Aires, also employing a crossed circulation; by Baschmakoff (1923) in Kazan, using the denervated salivary gland; by Hartman, McCordock and Loder (1923) in Buffalo, recording the changes in the completely denervated iris; by Anrep and Daly (1925) in London, studying the heart outside the body, but connected with the abdominal aorta and inferior vena cava; and by Kodama (1923), now supported by Sugawara,

Watanabé and Saito (1926), in Sendai, Japan, employing Stewart and Rogoff's own method. If support from anybody, anywhere, has been given to Stewart and Rogoff's observations and arguments, where? Their inference that the adrenin output continues at a uniform rate, unmodified by reflex stimulation and asphyxia, is wholly contradicted by experiments. On the other hand the concordant experience of investigators, derived from a variety of methods, justifies confidence in the response of the denervated heart as a sign of an increased discharge from the adrenal medulla.

The denervated heart is made to beat faster by increased adrenal, hepatic and thyroid secretion. When these sources of humoral stimulating factors are excluded the amount of acceleration in repeated extreme tests is slight (Cannon, Lewis and Britton, 1926). In order to observe the influence of any one of these humoral accelerators, therefore, it is only necessary to exclude the other two. Thyroid innervation through sympathetic connections is abolished by removal of the stellate ganglia (a part of the routine in denervating the heart). The discharge of the hepatic cardio-accelerating substance is excluded by severing the nerves on the duodeno-hepatic artery (Cannon and Uridil, 1921; Cannon and Griffith, 1922). Then medulliadrenal secretion alone remains to produce a faster heart rate. That this is true can readily be proved by removal of the right adrenal and denervation of the left, whereupon the acceleration ceases.

The lasting preparation of the denervated heart has important advantages over other methods. From it an almost continuous graphic record can be obtained, revealing the latent period of the response after any procedure, the moment of greatest activity, and its gradual subsidence. Instead of an observation on a single sample of blood, taken somewhat blindly, without knowledge of its relation to the total reaction, the total reaction can itself be followed; and not one experiment only, but as many as desired, in the same animal, all without the possibility of the profound depression which may attend anesthesia (cf. Cannon and Rapport, 1921, table 3; and Sugawara, Watanabé and Saito, 1926). And furthermore, after various tests have been repeated with the adrenal connections intact, the same tests on the same animal, after the adrenals have been inactivated, may be repeated as many times as before if that is desired, or until the proof is clear that the previous acceleration was due to a greater output of adrenin. The importance and the great advantage of making these observations on healthy vigorous animals, free from the influence of narcotics, and of controlling the observations on the same animals, cannot be emphasized too strongly.

The question arises as to the degree of reliance which may be placed on increases in the rate of the denervated heart in judging how widely the discharge of adrenin varies. That the preparation is a wholly satisfactory

zero instrument, showing whether the adrenin output is increased or not, has been proved in earlier experiments in this series (Cannon and Rapport, 1921; Cannon and Carrasco-Formiguera, 1922). But in those experiments the animals were quiet, under anesthesia. In active, vigorous animals can it reasonably be used to indicate differences in secretion? In answer to this question the following facts are pertinent. 1. Mere increase in the *amount* of adrenin-containing blood passing through the coronary vessels of the denervated heart in a given time, as caused by rise of blood pressure (see Markwalder and Starling, 1913), does not alter noteworthily the rate of beat. This was shown by Cannon and Rapport (1921, fig. 2) and confirmed by Anrep and Daly (1925). 2. Within limits the denervated heart responds to increasing *concentrations* of adrenin in the blood by an increasingly faster rate. Cannon and Rapport (1921) showed that repeated equal intravenous doses of adrenalin produce the same or nearly the same increments of heart rate (in an animal with the circulation confined to the trunk vessels) and that as the doses are made larger the cardiac accelerations become greater. A similar relation between adrenin concentration and the rate of beat was demonstrated by Anrep and Daly (1925) in the heart-lung preparation. 3. The circulation rate is normally faster during muscular activity than during rest (see Wiggers, 1923). In part this is due to a larger return of blood to the heart in a given time, and in part to a more rapid heart rate. The operation for denervating the heart leaves unchanged the factors which augment the return of blood from the periphery during muscular exertion. The isolated heart will put forth what it receives (cf. observations on the heart-lung preparation, Patterson and Starling, 1914). Therefore, during and immediately after activity there will be a faster circulation rate, even though the heart is not accelerated. According to Anrep and Daly (1925) variations in the circulation rate do not influence to an important degree the effect of adrenin on the rate of the isolated heart. Let us make two assumptions: *a*, that Stewart and Rogoff's conclusion is correct, i.e., that adrenin is secreted at a uniformly constant rate, not affected by afferent stimulation, asphyxia or emotional excitement; and *b*, that the secreted adrenin is destroyed as it passes through the systemic blood vessels (Elliott, 1905). Then, doubling the volume flow of blood would halve the concentration of adrenin. Anrep and Daly found that a change from 1 part of adrenin in 75 millions (approximately the ratio determined by Cannon and Rapport, 1921, in undisturbed experimental conditions) to 1 part in 150 millions lessened the increase of heart rate by only 6 beats per minute—the rate rose from 144 to 168 instead of from 144 to 174. Even when the concentration was reduced to one-fourth, which would result from quadrupling the volume flow, the effect was still not very great—144 to 162 instead of 144 to 174.

Although the faster circulation rate of muscular activity is not important

in reducing the influence of adrenin on the heart, it clearly does not favor a faster heart rate. It could do so only if the second of the two assumptions made above is wrong, i.e., only if the secreted adrenin is not destroyed. One might suppose that as the blood flows more rapidly the adrenin would pass, still potent, from the arteries to the veins, and that, thus accumulating and becoming more and more concentrated, it would cause a more rapid pulse. Invalidating that explanation is our repeated experience that the maximal effect appears promptly, within 20 or 30 seconds after a struggle, and thereupon the effect, instead of becoming larger, as would result from increasing accumulation, gradually becomes smaller. The facts, therefore, are opposed to the view that concentration of adrenin rises because it is not destroyed.

Since adrenin does not accumulate in the blood by escaping destruction, the faster heart rate after struggle leaves no other inference than that it is due to increased concentration resulting from increased discharge. Thus the first of the two assumptions made above, i.e., the correctness of Stewart and Rogoff's claim that the discharge is constant and unvarying, is shown to be unwarranted.

On the basis of the foregoing considerations it is possible to go still further. The minute-volume flow of blood rises gradually as exertion becomes gradually more intense (Douglas and Haldane, 1922). Thus the cardiac acceleration would become gradually slower if the adrenin output were constant. In fact, however, as activity becomes more vigorous the denervated heart beats gradually faster. The faster beat, then, occurs in spite of a faster circulation rate, and becomes still more rapid as the circulation rate rises still higher. In other words, as other conditions become less and less favorable to a quickening of the heart beat by an increased secretion of adrenin, the evidence for increased secretion becomes more and more pronounced. We see no other conclusion to draw from these observations than that as an animal manifests greater activity the adrenin output is greater. These points will be illustrated as the results of our experiments are examined.

Results. Three types of activity have been studied by us as associated with increased secretion from the adrenal medulla—minor movements with little or no affective accompaniment; emotion, with little or no movement of skeletal muscles; and a combination of the two in vigorous activity with excitement. The rate of the denervated heart was augmented in each of these states, but to different degrees.

In order to have some indication of the relative amount of cardiac acceleration under various circumstances it was necessary to establish a basal or standard rate. This was done by bringing the animal to a calm, quiet state while resting on a cushion held in the lap. The cat usually lay comfortably on one side, and without protest permitted the receiving

tambour to be held against the chest wall where the cardiac impulse was most prominent. The heart rate first recorded, after the animal with active adrenals was placed on the cushion, was regularly faster than it was later, for reasons which presently will be understood. Usually within five or ten minutes, if the animal remained quiet, the rate fell (the average fall was 16 beats) until it reached a constant low level. This was the basal rate. The changes of rate due to the various types of action mentioned above were referred back to the basal for comparison.

The effect of minor movements, without notable emotion. The observations of Hartman, Waite and Powell (1922) had furnished evidence that adrenal secretion is increased during muscular exercise. We were surprised to note, however, that even slight motions, such as extending the legs ("stretching"), or turning the body, or lifting the head, were attended by increase of heart rate. We thereupon induced such minor activities by lowering the cushion to the floor and letting the cat walk for a minute or two. The animal was then picked up gently, replaced on the cushion, and the heart rate recorded as soon as possible. In table 1 and in figures 1 and 2 are displayed results of these experiments.

Examination of table 1 shows that, with the exception of two instances in which the heart rate increased 18 and 24 beats, slight spontaneous activity, while the animal with intact adrenals was resting on the cushion, was followed by an increase of rate between 5 and 10 beats per minute. This result might be regarded as accidental, if it had not occurred regularly under the circumstances mentioned, and if it had not failed to occur after the adrenals were inactivated (see table 1).

When the animals were allowed to walk, the acceleration was greater. In one instance the heart beat was faster by 38 beats after a walk of two minutes, and in four other of the 14 observations the rise was above 20. These increases were unusual, however, and may have resulted from disturbance when the animals were picked up again in order to record their cardiac pulsations. As a rule the walking raised the rate between 10 and 20 beats. Cat 38 consistently showed large increases, and on four occasions the rise averaged 27 beats per minute. After inactivation of the adrenals the acceleration either failed to occur or was small (see table 1 and fig. 1).

The average cardiac acceleration in 27 observations on the effect of minor movements, without notable emotion, was 15 beats per minute. The variations ranged between 6 and 38 beats. After the adrenals were inactivated the average acceleration in 23 tests under the same conditions was 1, with variations between −2 and 5 (see fig. 2).

The effect of emotional excitement, with slight muscular activity. This condition was induced in two ways: by bringing an aggressive dog near the cat while it was resting quietly on the cushion, and by fastening the cat

TABLE 1

Changes of rate of the denervated heart accompanying minor movements, without notable emotion

ADRENALS ACTIVE					ADRENALS INACTIVE				
Cat number, date	Basal heart rate, quiet on lap	Increase after slight activity on lap	Increase after brief walk	Remarks	Cat number, date	Basal heart rate, quiet on lap	Increase after slight activity on lap	Increase after brief walk	Remarks
(23)					**(23)**				
Jan. 21				Heart denervated	Feb. 11				Adrenals inactivated
Jan. 26	120	18			Feb. 17	88	0		
Jan. 27	116	8		Slight restlessness	Mar. 4	104	0		
Feb. 3	112	8							
(26)					**(26)**				
Mar. 2				Heart denervated	Mar. 25				Adrenals inactivated
Mar. 19	116		10	Walked 10 steps	Mar. 26	120		4	
Mar. 20	92	24		Played ½ minute	Mar. 30	108	0	0	Walked 1 minute
Mar. 20			24	Walked ½ minute	Apr. 1	104	0		
Mar. 22	90	8		Slightly restless					
(27)					**(27)**				
Mar. 3				Heart denervated	Apr. 6				Adrenals inactivated
Mar. 13	144	6		Restless	Apr. 7	94		0	Walked 2 minutes
Mar. 18	120		20	Walked 5 steps	Apr. 10	108		0	Walked 4 minutes
Apr. 6	96		38	Walked 2 minutes	Apr. 13	92	0		
					Apr. 16	118		2	
(33)					**(33)**				
Mar. 23				Heart denervated	Apr. 30				Adrenals inactivated
Apr. 12	122		10	Walked 1 minute	May 1	128	−2		Slight activity
Apr. 16	118	6		Restless	May 2	128	0		
Apr. 16			16	Walked 2 minutes					

(38)					(38)				
May 12				Heart denervated	June 4				Adrenals inactivated
May 27	150	6		Stretching, pawing	June 5	120	2	4	
May 27			18		June 7	144	−2		Stood up, stretched limbs
June 1	118	10	26		June 7			2	
June 2	128		34	Walked ½ minute					
June 4	130		30						
(46)					(46)				
June 11				Heart denervated	June 24				Adrenals inactivated
June 19	128	10	16		June 25	112	0		Turned over
June 21	124	6		Stretched limbs few times	June 25			5	
June 21			14		June 28	100	2		
(49)					(49)				
June 10				Heart denervated	June 24				Adrenals inactivated
June 19	118	10	14		June 25	114	0		Stood up, prepared to jump from lap
June 21	100	6		Playful					
June 21			10		June 25			4	
					June 28	101	1		Walked 1 minute
					June 28			3	

back downward in a comfortable holder. Usually when the dog approached the resting cat and began to bark, the cat showed some or all of the following signs of excitement—dilatation of the pupils, erection of hairs on the tail and back, retraction of the ears, baring of the teeth,

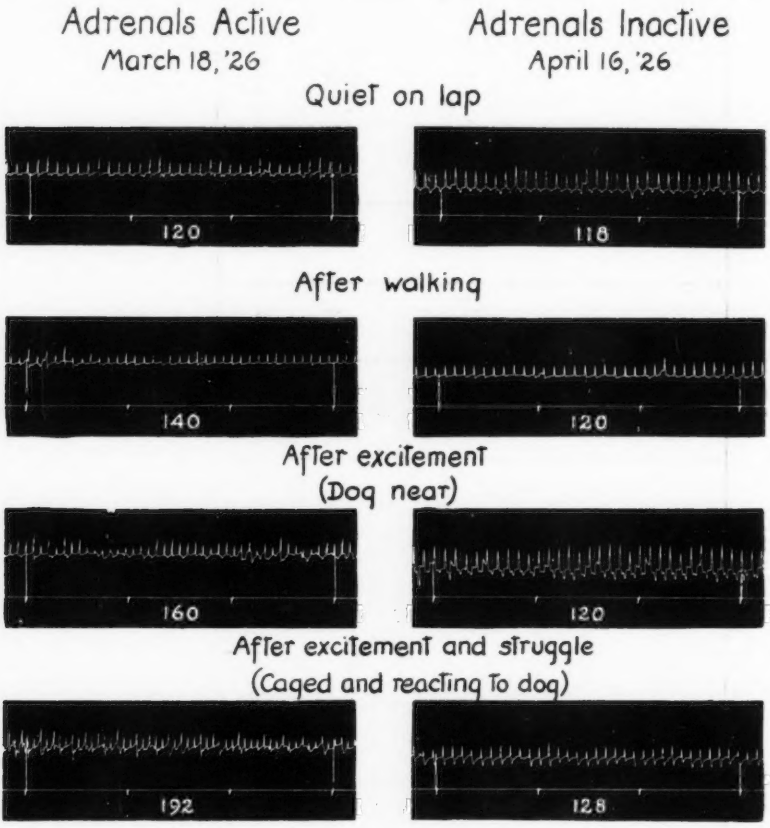

Fig. 1. Original records of the rate of the denervated heart of cat 27 taken before (on March 18) and after (on April 16) medulliadrenal secretion was excluded (on April 6). Time in 5-second intervals. The basal rate with the animal quiet on lap is to be compared with the rate after walking, after being excited by the presence of a dog, and after caged and reacting to a barking dog.

hissing, snarling, spitting, and striking at the dog with one paw or sometimes with both paws in rapid succession, with claws protruding. When the animal was placed in the holder, motion of the neck was limited by two

upright rods which were closed above by a cross-bar; the fore legs were restrained loosely in thongs which passed through holes in the side of the holder; the hind legs were not fastened, but were grasped between the

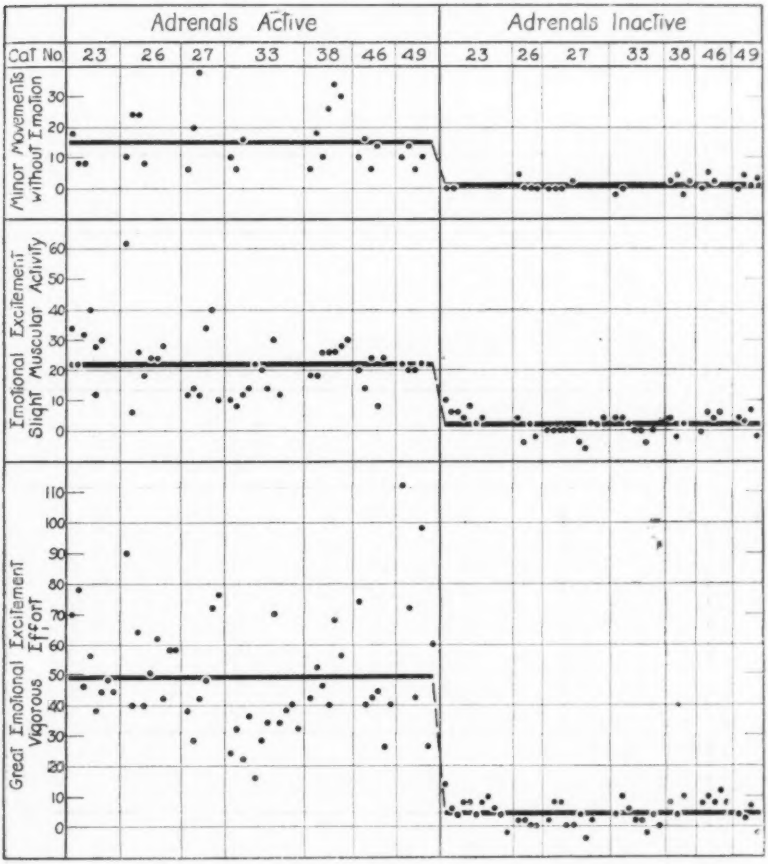

Fig. 2. Graphic record of the results presented in tables 1, 2 and 3. The dots represent increases of rate of the denervated heart above the basal level that followed *a*, minor movements without notable emotion; *b*, emotional excitement with slight muscular activity, and *c*, great emotional excitement with vigorous effort, before and after inactivation of the adrenal medulla. The wide line indicates in each condition the average of the changes of heart rate.

fingers of one of the observers. The usual behavior of the animal under these circumstances was characterized by minor indications of disturbance—uneasy twitching of the tail, occasional mewing, and turning of the

TABLE 2

Changes of rate of the denervated heart accompanying emotional excitement, with slight muscular activity

ADRENALS ACTIVE					ADRENALS INACTIVE				
Cat number, date	Basal heart rate, quiet on lap	Increase after excited by dog	Increase after fastened to holder	Remarks	Cat number, date	Basal heart rate, quiet on lap	Increase after excited by dog	Increase after fastened to holder	Remarks
(23)					**(23)**				
Jan. 21				Heart denervated	Feb. 11				Adrenals inactivated
Jan. 27	116		34		Feb. 12	76		10	
Jan. 29	104	22		Cat saw dog at door, entering room	Feb. 13	80		6	
					Feb. 15	78		6	
Jan. 29			32		Feb. 17	88		4	Active resistance on being tied to holder
Jan. 29	104	40		Dog brought near cat					
Feb. 3	112	28		Cat hissed, tail hairs up	Feb. 19	86		8	
Feb. 3			12		Feb. 23	108	2		Cat hissed, growled, showed teeth, lashed tail, hairs up
Feb. 5	90		30	Slight spontaneous activity	Feb. 23			4	
(26)					**(26)**				
Mar. 2				Heart denervated	Mar. 25				Adrenals inactivated
Mar. 8	146		62		Mar. 27	104		4	
Mar. 10	128	6		Very slight reaction to dog	Mar. 30	108	−4		Cat hissed, ears back, teeth showing, no major movements
Mar. 10			26						
Mar. 16	120		18		Mar. 30			2	
Mar. 18	98	24		Dog 6 meters from cat, no movement of cat	Apr. 1	104		−2	
Mar. 19	116		24						
Mar. 25	104		28						

(27)					(27)			
Mar. 3				Heart denervated	Apr. 6				Adrenals inactivated
Mar. 8	144		12		Apr. 7	96		0	
Mar. 10	148		14	Poor reaction; recovery from operation slow	Apr. 8	104	0	0	Struck at dog 3 times, showed teeth
Mar. 13	140		12	Very quiet on holder	Apr. 10	100	0		Hissed and struck at dog
Mar. 16	130		34		Apr. 10			0	
Mar. 18	120	40		Cat jumped on floor, walked few steps, then retaken	Apr. 12	112	−4		Hairs erect, hissing
					Apr. 13	106	−6		Marked reaction
					Apr. 16	118	2		Hissing, striking, teeth showing
Apr. 6	92		10						
					Apr. 17	116		2	
					Apr. 20	124		4	Vigorous struggle on holder
(33)					(33)				
Mar. 23				Heart denervated	Apr. 30				Adrenals inactivated
Apr. 10	116		10	Respiratory infection, cat inactive	May 1	124		4	
					May 2	128	4		Excited 2 minutes, hissing, striking, hairs up
Apr. 12	122	8		Crouching, no general movements					
Apr. 12			12		May 2			2	
Apr. 16	118	14		Hairs up, teeth showing, hissing	May 3	124		0	
					May 5	122		0	
Apr. 16			22		May 7	122		−4	Struggling about 15 seconds
Apr. 26	132		20						
Apr. 28	120	14		Crouching, hissed twice, showed teeth	May 8	130		0	
Apr. 28			30						
Apr. 30	124		12						

TABLE 2—*Concluded*

ADRENALS ACTIVE					ADRENALS INACTIVE				
Cat number, date	Basal heart rate, quiet on lap	Increase after excited by dog	Increase after fastened to holder	Remarks	Cat number, date	Basal heart rate, quiet on lap	Increase after excited by dog	Increase after fastened to holder	Remarks
(38)					(38)				
May 12				Heart denervated	June 4				Adrenals inactivated
May 24	144		18		June 5	120		4	
May 27	150	18		Cat very excited	June 7	144		−2	Crying and jerking on holder
May 27			26						
May 28	130		26		June 9	138		2	
June 2	128	26		Withdrew from dog, but hairs erect					
June 2			28						
June 4	130		30						
(46)					(46)				
June 11				Heart denervated	June 24				Adrenals inactivated
June 19	128		20		June 25	112		6	
June 21	124	14		Hairs erect, no aggression shown	June 28	100	0	4	
					July 2	106		6	Vigorous resistance on holder
June 21			24						
June 24	126	8		Moderate reaction to dog					
June 24			24						
(49)					(49)				
June 10				Heart denervated	June 24				Adrenals inactivated
June 19	118		22		June 25	114		4	Very vigorous struggle
June 21	100	20		Hissing, striking, etc.	June 28	101	3		Good response when dog near
June 24	118	20		Dog afraid of cat, but cat showed defensive attitude	June 28			7	
					June 30	110		−2	
June 24			22						

head from one side to the other. At times the animal immediately resisted the strange position and the restraint, and struggled vigorously. This violent reaction we are not concerned with now; we are interested in what accompanies the little signs of excitement just mentioned, such signs as may be associated with complete cessation of gastro-intestinal movements (Cannon, 1911). In whatever manner the cat was excited the heart rate was recorded as soon as possible thereafter; within a minute after the dog had been taken away, and almost immediately after the cat was attached to the holder.

Reference to table 2 and to figures 1 and 2 shows the effects on the heart rate when emotional reactions occurred which were not attended by considerable contractions of skeletal muscles. Although the rise of rate may be slight when the cat is barked at by the dog, it is usually greater than when there is motion without excitement,—ranging between approximately 15 and 30 beats per minute instead of the 10-to-20-beat acceleration recorded after walking. It is noteworthy that when the cardiac response was small there was only a minor reaction to the presence of the dog (e.g., no. 26, March 10), and when the response was large the reaction was much more marked, with evidence of sympathetic activity in the standing hairs and the dilated pupils (e.g., no. 23, February 3).

The heart was made to contract somewhat faster by attaching the animal back-downward to the holder. In these circumstances the outward signs of disturbance varied considerably. Some animals took the experience relatively calmly from the beginning (cf. no. 27, March 8, 10, 13). Others were excited at first, and as they gradually became accustomed to the unnatural position were less perturbed by it (cf. no. 23, January 27, 29 and February 3; also no. 26, March 8, 10, 16). Corresponding with these variations of emotional reaction were the degrees of cardiac acceleration. The figures 10 to 35 cover most of the increments of heart rate that were observed, though in cat 26 the first confinement in the holder called forth a rise of 62 beats per minute.

The above increments in heart rate occurred when the adrenal medulla was undisturbed; in striking contrast were the cardiac responses after medulliadrenal inactivation. The animals were repeatedly subjected to the same conditions as before. The obvious reactions were precisely those which had previously been seen—hissing, growling, baring of the teeth and erection of the hairs when the dog approached the cat or barked and made aggressive motions; and twitching of the tail, mewing, and signs of uneasiness when the cat was fastened in the holder. Yet, as shown by the results in table 2 and as illustrated in figure 1, the heart rate, instead of rising sharply 30 or 35 beats per minute, decreased somewhat (see no. 27, April 12, 13), or remained unchanged (see no. 33, May 3, 5, 8), or showed a minor acceleration (see no. 23, February 12–23).

The average increment of cardiac pulsation in the 45 observations on the effect of emotional excitement, with only slight muscular activity, was 22 beats per minute. After the right adrenal gland had been removed and the left denervated the average increment in 39 observations was 1.8 if the decreases of rate are subtracted from the increases, and 2.5 if the decreases are given no value (see fig. 2 in which the average is marked at 2).

In connection with the effects of excitement, accompanied by little or no activity of skeletal muscle, some incidental observations on cardiac reactions of the cat in the presence of mice and rats are pertinent. Cat 26 had a basal rate of 92 at 9:55, March 20. A "catnip mouse" was brought near. The cat stood up on the cushion and played with the "mouse" for about 30 seconds. It was then removed. At 10:00 the heart rate was 116; at 10:04, 112; and at 10:06, 108. Again at 10:25, when the basal rate was 96 as the cat was lying on the cushion, a small cage containing a white mouse was brought near. The cat turned his head to one side, kept his eyes on the mouse as it moved about, and, though not rising or moving on the cushion, reached towards the mouse with a fore paw. This lasted from 10:27 to 10:29 when the cage was taken away. At 10:29 the heart rate had risen 20 beats (to 116); at 10:31, it was 116; at 10:34, 100; and at 10:36, 96.

Similar responses were observed in cat 27. On April 6, at 12:15, while the cat was quiet on the cushion the heart rate was 96. At 12:15½ a small cage containing a white rat was brought near. The cat showed some signs of interest, but did not make gross movements. At 12:17 the heart rate was 112. The rat was then taken from the cage and held near the cat. He lunged towards it, and not reaching it, sniffed (12:19). The rat was removed. At 12:20 the heart rate was 116; at 12:22, 108; at 12:24, 98. The catnip mouse was then dangled above the cat, which caught it and bit it. At 12:29, the heart rate was 114; at 12:30, 112; and at 12:33, 104. As shown by the control observations detailed in table 2 and figure 1, the denervated heart, after adrenal inactivation, would accelerate only 1 or 2 beats per minute, instead of 20 or more beats as above, in response to such minor excitement.

The effect of great emotional excitement, with vigorous muscular effort. Two methods were employed to bring about energetic movements combined with extreme emotional excitement. The cat was shut in a wire cage where it was barked at and attacked by a dog outside; or it was fastened into the holder, as described above, where it struggled either spontaneously or because of brief pressure near the root of the tongue. The experience in the cage seldom continued more than a minute. Of course the cat could not be injured inside its wire protection. As the dog rushed about the enclosure, however, the cat shifted its position quickly, keeping the enemy in view, now crouching, now counter-attacking, hissing, spitting, growling,

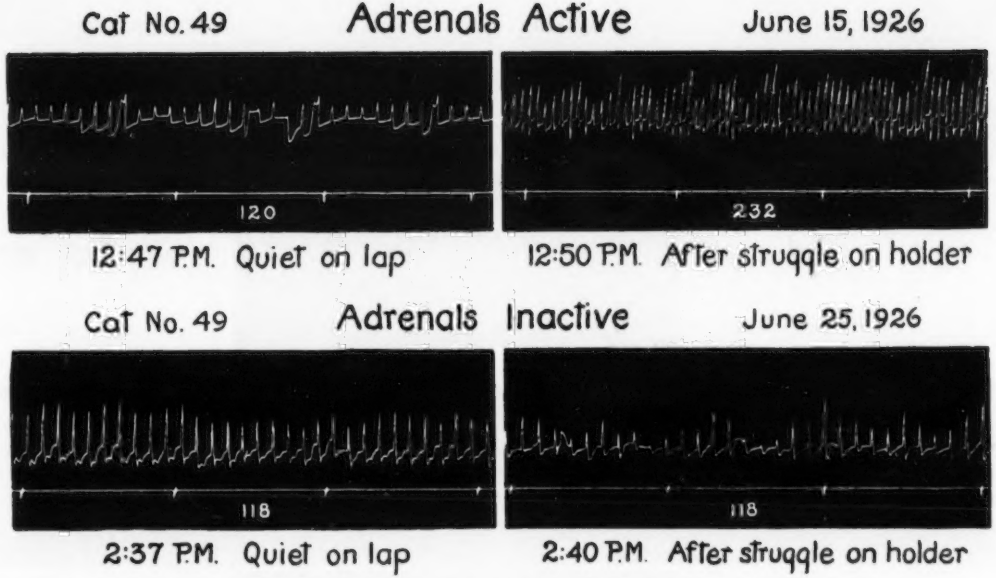

Fig. 3. Original records of cat 49 to show a maximal increase of rate of the denervated heart (112 beats per minute) that resulted from vigorous struggle, June 15, when the adrenals were active and the animal was fastened to the holder, and the absence of any increase, when, on June 25, with adrenals inactive, a correspondingly vigorous struggle was induced in the same manner. The interval between the records in each condition was 3 minutes. Time marked in 5 seconds.

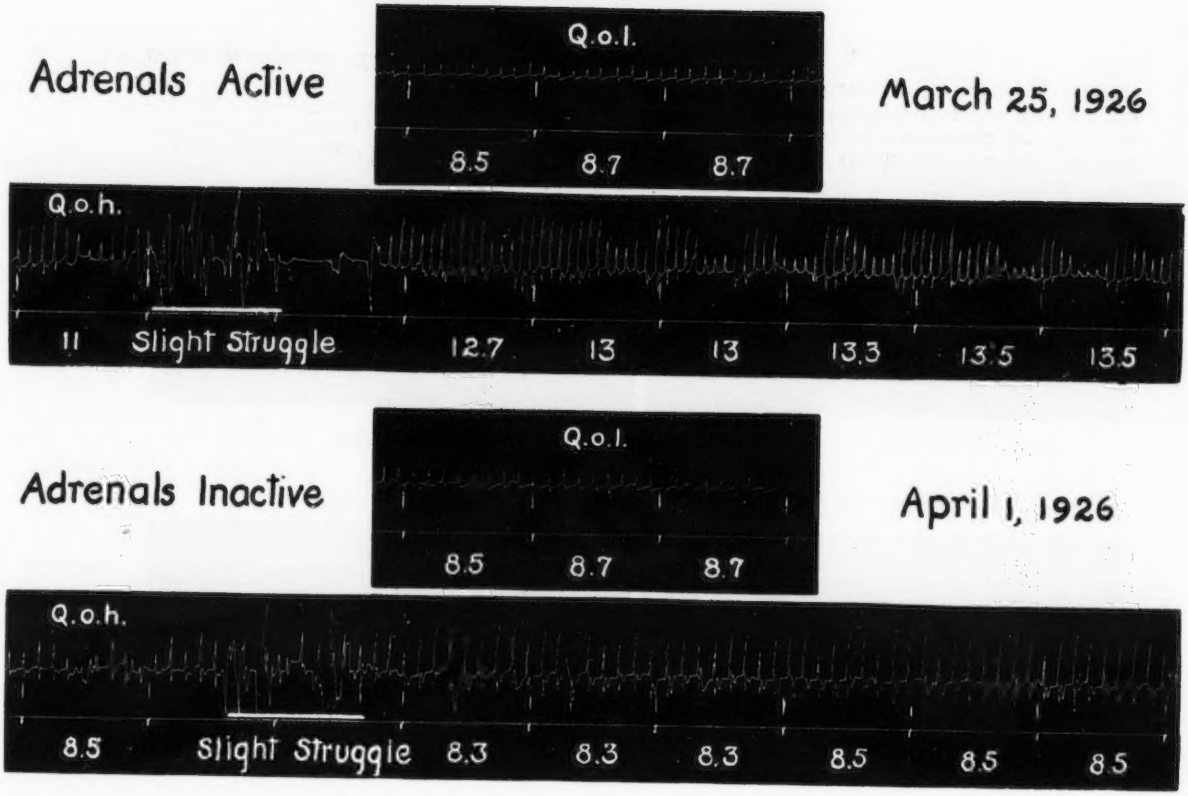
Adrenals Active
Q.o.l.
March 25, 1926
8.5 8.7 8.7
Q.o.h.
11 Slight Struggle 12.7 13 13 13.3 13.5 13.5
Adrenals Inactive
Q.o.l.
April 1, 1926
8.5 8.7 8.7
Q.o.h.
8.5 Slight Struggle 8.3 8.3 8.3 8.5 8.5 8.5

Fig. 4

striking, with ears drawn down, teeth showing, back arched, pupils widely dilated, hairs on the back erect and tail enormous. Immediately after the "fight" ended, the cat was removed from the cage, placed on the cushion and the heart rate recorded. The struggle in the holder has already been described (Cannon, Lewis and Britton, 1926). It was usually violent while it lasted—characterized by jerking the fore legs in the thongs, pulling and pushing the hind legs in the assistant's fingers, snarling, biting at the cross-bar and exhibiting every sign of resentful rage—but it rarely lasted more than 5 or 10 seconds and was usually followed by a period of quiet during which a good record of the heart rate could at once be obtained.

The results recorded in table 3 and illustrated in figures 1, 2, 3 and 4 reveal immediately how greatly the heart is accelerated when, with adrenals intact, vigorous action or struggle is combined with excitement. If the early observations on no. 33 (while it had a respiratory infection) are excluded, the minimal increase of rate was 26 beats per minute, the maximal 72, after the cat had been caged and barked at by the dog. It was not possible to obtain an immediate record of the pulse, and probably the maximal was higher than that set down in the table. In round numbers the increment of rate may be considered conservatively as ranging usually between 30 and 50 beats per minute—the average increase in 18 observations was 38.

A still greater response than this is usually seen when the cat struggles in the holder (see figs. 3 and 4). Again with no. 33 excluded, the increases of heart rate varied between 38 and 112, with eleven observations between 60 and 100. A rough statement of the extent of variation of the increment due to a mad and violent effort would be 40 to 80 beats per minute—26 of the 35 observations lie within that range. After adrenal inactivation the maximal rise of rate was 14 beats in two tests; in all the other 40 tests 8 beats or fewer were usually recorded, and in a few instances the heart actually worked more slowly after the struggle (see fig. 4).

The average speeding up of the denervated heart in 53 observations on the effect of great emotional excitement attended by vigorous muscular effort was 49 beats per minute. In 43 observations after adrenal inactivation the average was 4.5 if the decreases of rate are subtracted from the

Fig. 4. Original records of cat 26, to show the short latent period and the gradual development of the maximal response. On March 25 with the adrenals active the basal rate (quiet on lap—*q. o. l.*) was 104 beats per minute, and when quiet on the holder (*q. o. h.*) the rate was 132; after a slight struggle (shown by irregularities in the record) it rose to 152.5 within fifteen seconds, and at the end of thirty seconds after the start of struggle it was 162—an increase of 58 beats per minute. The adrenals were inactivated on March 25, after the foregoing records were made. On April 1, with the same basal rate (104), the rate *q. o. h.* was 102, and a similar slight struggle was followed by a drop to 99.5 and then a gradual rise to 102.

TABLE 3

Changes of rate of the denervated heart accompanying great emotional excitement, with vigorous muscular effort

ADRENALS ACTIVE					ADRENALS INACTIVE				
Cat number, date	Basal heart rate, quiet on lap	Increase after caged and excited by dog	Increase after struggle on holder	Remarks	Cat number, date	Basal heart rate, quiet on lap	Increase after caged and excited by dog	Increase after struggle on holder	Remarks
(23)					(23)				
Jan. 21				Heart denervated	Feb. 11				Adrenals inactivated
Jan. 26	120		70	Struggle while held on lap only	Feb. 12	76		14	Condition good
					Feb. 13	80		6	
Jan. 27	116		78		Feb. 15	80		4	20 seconds' vigorous struggle
Jan. 29	104	46		Back and tail hairs rough, good reaction					
Jan. 29			56		Feb. 17	88		8	
Feb. 3	112	38		Hissing, snarling, tail very big	Feb. 19	86		8	
					Feb. 23	108	4		Cat struck at dog, tail enormous
Feb. 3			44						
Feb. 5	90		48		Feb. 23			8	
Feb. 9	92		46		Feb. 25	78		10	
					Mar. 1	114		6	Struggling 15 seconds
					Mar. 4	104		4	
					Mar. 8	114		−2	

(26)					**(26)**				
Mar. 2				Heart denervated	Mar. 25				Adrenals inactivated
Mar. 8	146		90		Mar. 27	104		2	
Mar. 10	128	40		Hissing, tail and back hairs up	Mar. 30	108	2		Ears back, teeth showing, hissing, spitting, striking
Mar. 10			64						
Mar. 13	120		40		Mar. 30			0	
Mar. 15	120		50	Vigorous spontaneous struggle	Apr. 1	104		−2	No increase in rate after several struggles
Mar. 18	98	62		Striking at dog, hissing, hairs up					
Mar. 19	116	42		Usual reaction to dog					
Mar. 19			58						
Mar. 25	104		58						
(27)					**(27)**				
Mar. 3				Heart denervated	Apr. 6				Adrenals inactivated
Mar. 8	144		38		Apr. 7	94		2	
Mar. 10	148	28		Hissing, growling, spitting, striking, tail and back hairs up	Apr. 8	104	8		Very reactive, hissing, striking, hairs up
					Apr. 8			8	
Mar. 13	140		42		Apr. 10	100	0		Cat very aggressive, marked reactions
Mar. 16	130		48						
Mar. 18	120	72		Vigorous reaction	Apr. 10			0	
Apr. 6	96		76		Apr. 16	118	10		
					Apr. 17	114		4	
					Apr. 20	124		−4	Vigorous struggle 20 seconds
					Apr. 28	112		2	

TABLE 3—*Concluded*

ADRENALS ACTIVE					ADRENALS INACTIVE				
Cat number, date	Basal heart rate, quiet on lap	Increase after caged and excited by dog	Increase after struggle on holder	Remarks	Cat number, date	Basal heart rate, quiet on lap	Increase after caged and excited by dog	Increase after struggle on holder	Remarks
(33)					(33)				
Mar. 23				Heart denervated	Apr. 30				Adrenals inactivated
Apr. 1	120	24	32	Cat has rhinitis, apathetic	May 1	124		4	
					May 2	128	10		Cat very excited, usual display
Apr. 10	116	22	36	Health improved					
Apr. 12	122	16	28	Moderate excitement only	May 2			6	
Apr. 16	118	34	70		May 3	124		2	
Apr. 26	132		34		May 5	122		−2	
Apr. 28	120	38	40	Usual reaction	May 7	122		4	Vigorous struggle
Apr. 30	124		32		May 8	130		0	
(38)					(38)				
May 12				Heart denervated	June 4				Adrenals inactivated
May 27	150	42	52		June 5	120		8	
May 28	130		46		June 7	144		4	Struggled several times
June 2	128	40		Cat not aggressive, crouching, ears back	June 9	138		10	
June 2			68						
June 4	130		56	Slight spontaneous struggle					

(46)					(46)				
June 11				Heart denervated	June 24				Adrenals inactivated
June 15	136		74	Moderately active only	June 25	112		8	Struggling 15 seconds
June 19	128		40		June 28	100	10	8	
June 21	124	42		Appeared unafraid of dog	June 30	96		12	
June 21			44		July 6	114		8	Vigorous reaction
June 24	126	26	40						
(49)					(49)				
June 10				Heart denervated	June 24				Adrenals inactivated
June 15	120		112	Spontaneous struggle	June 25	114		4	
June 16	126		72		June 28	101	3	7	Usual reactions
June 21	100	42		Brisk reaction to dog	June 30	110		−2	
June 21			98						
June 24	118	26	60						

increases, and 5 if the decreases are counted as zero (see fig. 2 in which the average is marked at 5).

Related to the foregoing increments of heart rate are results noted when a stomach tube was passed for the purpose of introducing cold water (see Cannon, Querido, Britton and Bright, 1926). Usually this was accompanied by some resistance on the part of the animal. The pulse was raised to varying degrees; in one animal with a respiratory infection the increase was only 22 beats, in two others it varied from 32 to 56, and in a fourth the increases were 76, 116, 124 and 132 on four different occasions. After exclusion of medulliadrenal influence the maximal increase under the same conditions in the same animals was 10 beats per minute.

Struggle in the holder offers a favorable condition for determining the latent period of the response to excitement, for the position of the animal permits a record of the heart as soon as the struggle ceases, and, as stated above, the effort is usually brief. Acceleration of the denervated heart from reflex medulliadrenal secretion begins in about 8 seconds and reaches a maximum in about 35 seconds (Cannon and Uridil, 1921). As shown in figure 4, the rise of heart rate after struggle takes a similar course if the adrenal glands are intact, and fails if they are not functioning.

Persistence of the excited state after disappearance of the exciting condition. A striking feature of the influence of emotional disturbance on gastric secretion is the continued inhibition, lasting long after the apparent excitement has subsided. For example, Bickel and Sasaki (1905) noted that, whereas sham feeding of a dog for five minutes caused during the following twenty minutes a flow of 66.7 cc. of gastric juice, sham feeding for the same time, but after five minutes of exciting experience with a cat, caused during the next twenty minutes a flow of only 9 cc. of a juice rich in mucus; and when the excitement followed the sham feeding the inhibition was nearly complete. It was this persistence of the visceral disturbance after the occasion for it had passed that led to the first observations on the emotional secretion of adrenin, for it had seemed possible that the lasting effect might be due to circulating adrenin which would continue by chemical action the changes initiated by nerve impulses (see Cannon, 1915, p. 40). The common experience that calmness and serenity are not restored at once after a period of great emotional stress, that a state of uncontrollable agitation persists, was related to that suggestion. It was a matter of considerable interest, therefore, to learn whether adrenal secretion did in fact go on for some time after the obvious excitement had ceased—an enquiry made readily possible by study of the denervated heart.

The most favorable condition for learning fairly the influence of strong emotion on the course of medulliadrenal secretion was that of the caged cat attacked by the dog. The signs of emotional upset were maximal, the big muscles were not used so much as in the struggle on the holder, and

as soon as the brief fray was ended the cat could be placed on a cushion and the heart beat could be recorded at regular intervals without any further annoyance. As illustrated in figure 5, a high heart rate is found immediately after the removal of the excited cat from the cage. The increased rate rapidly falls, but not sharply to the basal level. That level may not be reached for twenty or twenty-five minutes after an exciting experience which itself lasts only a minute. After the adrenal glands were inactivated, even though the experience in the cage was

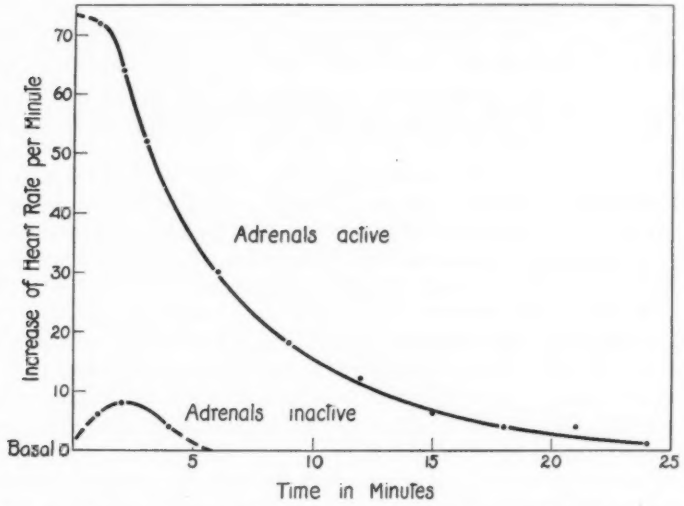

Fig. 5. Long persistence of the faster heart rate, when the adrenals were active, after the animal (cat) was excited by a barking dog for one minute. Only slight increase of heart rate and its relatively rapid subsidence, after adrenal inactivation, though the cat was excited twice as long as before. In both instances the animal was promptly removed from the cage after the excitement, and during the period of recovery rested quietly on a cushion.

extended to two minutes, the increment of heart rate, as shown in figure 5, was 8 beats as compared with 72 beats before the inactivation, and the basal was recovered within ten minutes as compared with twenty-five minutes previously.

Five animals which had struggled on the holder for less than ten seconds were thereafter studied while quiet on a cushion. After ten minutes the increase of heart rate was still 12 or more beats per minute in all these cases, and after twenty minutes it was still 2 to 6 beats above the basal in three of them.

The return to the former quiet state occurs sooner when the adrenal response is less intense. For example, in cat 26, an excitement which caused an increase of 40 beats was recovered from in ten minutes, whereas one which caused an increase of 62 beats had not completely subsided at the end of fourteen minutes (see table 4).

Discussion. That the faster beating of the denervated heart in the circumstances described above was due to increased adrenal secretion is proved by a variety of considerations. It could not have been due to increased temperature, for the minor movements and the almost pure visceral responses would not be associated with noteworthy quick heat production; and even where the contractions of skeletal muscles were maximal, as in the struggle on the holder, they were brief, and though they might by raising the temperature of the blood 1° cause the heart to accelerate by 6 to 12 beats per minute (Knowlton and Starling, 1912), they could not possibly account for such increments as more than 100 beats per minute. Furthermore, we would emphasize again the points *a*, that the heart was made to pulsate most rapidly when muscular exertion was most prominent in the reaction; *b*, that under this condition the circulation would be most rapid; *c*, that the faster circulation would dilute a uniformly secreted adrenin; *d*, that such dilution would induce a slower heart rate, and therefore *e*, that the very much faster rate results from an increased adrenal secretion, overwhelming the factors which would diminish its effect by dilution. The foregoing considerations are fully supported by our control tests. These tests were carried out on the same animals that yielded the remarkable positive results presented above. The only difference between the original and the control observations was removal of one adrenal gland and denervation of the other in the latter circumstances. After this exclusion of the medulliadrenal agency the faster pulse wholly failed to occur or was so slight as to be explicable by a rise of temperature. These results fully confirm the earlier evidence, brought forward in these laboratories and elsewhere, that as a part of the reaction of the organism in great excitement there is a striking increase of the discharge of adrenin from the adrenal medulla.

One of the most surprising of our results was the involvement of the adrenal medulla in merely slight muscular activities. Doubtless the reason why the heart rate was not basal when the cat was taken from the floor and set on the cushion was that the animal had just before been moving about, or had resisted being picked up; the average drop in heart rate was about the same as the increase caused by walking. This close association of adrenal secretion with movement brings up again the question of its service in the organism. Evidence obtained in these laboratories that adrenin is able to produce a rapid recovery of the capacity of fatigued muscle to respond to stimulation (see Gruber, 1914)—an effect interestingly

related to recent testimony as to the beneficial influence of sympathetic impulses on fatigued muscle (see Orbeli, 1923)—as well as considerations that fear and rage might be preparatory for flight or attack, had led to the inference that emotional stimulation of medulliadrenal secretion is favorable to prolonged muscular effort (Cannon, 1914). The increased secretion of adrenin accompanying incidental and routine muscular movements such as walking, its greater concentration in the blood during muscular exercise (see Hartman, Waite and Powell, 1922) and its abundant outpouring as a consequence of vigorous struggle emphasize the probable significance of adrenin for efficient use of muscles in the body. We are now endeavoring to discover what value adrenin may have in the course of continued physical exertion.

The results reported in table 3 prove that the output of adrenin is greatest when emotional excitement is combined with violent struggle. Un-

TABLE 4

Cases illustrating the slow recovery of the basal rate of the denervated heart after emotional excitement. During observation the animals were quiet on a cushion. The adrenals were active in all cases

CAT NUMBER	DATE, 1926	BASAL HEART RATE	INCREASE OF HEART RATE ABOVE BASAL AT STATED MINUTES AFTER CAT CAGED AND EXCITED BY DOG FOR 1 MINUTE											
			1 m.*	2 m.	4 m.	6 m.	8 m.	10 m.	12 m.	14 m.	16 m.	18 m.	20 m.	22 m.
26	Mar. 10	128	40	32	18	8	2	0						
26	Mar. 18	98	62	42	30		12	10	2	2				
26	Mar. 19	116	44	36	16		6		2	0				
27	Mar. 18	120	72	64	52	30		18	12	6	4		4	1
33	Apr. 16	118	34		18	10	4							
46	June 24	126	26				18		4					

* Approximately correct.

fortunately we have not been able to contrive an experiment in which medulliadrenal secretion can be estimated after great muscular effort without attendant excitement. The secretion incidental to vigorous activity and that resulting from the intense emotional state, therefore, cannot now be separated. As shown in table 2, however, the output is considerably less when the visceral manifestations of emotional stress are not accompanied by effort. These results coincide with those reported for pseudaffective medulliadrenal secretion; when the preparation was "quiet," though paws were wet with sweat and hairs were erect, the average rate of the denervated heart was 222, and when the preparation became active the rate rose to 252 (Cannon and Britton, 1925). Since increased secretion of adrenin is evidence for increased discharge of sympathetic impulses to the adrenal medulla, and since the sympathetic system is organized for

general and diffuse discharge (see Cannon, 1915, p. 26) even if the circulating adrenin did not have that effect, it follows that the bodily processes are much more profoundly altered in the full expression of emotion, i.e., in adaptive visceral changes *plus* vigorous effort, than in the preparatory visceral changes alone. This fact gives support to the suggestion made by Cannon and Britton (1925) that when limitation of the bodily disturbances is desirable, control of the controllable factors, the voluntary muscles, would minimize the effects of an emotional storm. It is clear that this new evidence modifies earlier advice, based on the theoretical desirability of completing a physiological process, that the bodily changes due to excitement should be "worked off" by strenuous exertion.

The results reported above offer another interesting hint. It is sound doctrine that the organism profits by the exercise of the functions of its parts. The deep stirring and unsettling of the routine of quiet existence, wrought by great excitement, may be as important for proper service of the viscera as is work for the proper service of muscles. One might argue, therefore, that occasional intense excitement is a physiological need. Strong physical effort, however, operating either directly *via* a larger sympathetic discharge or indirectly *via* increased output of adrenin, can influence the viscera perhaps nearly as well as excitement alone. Thus exertion is favorable to bodily welfare not only by bringing into use the muscular mechanisms, but also by requiring those internal adjustments, commonly the consequences of emotional excitement, which contribute to muscular efficiency.

The persistence of the visceral concomitants of excitement for some time after the stimulus for them has ceased to act, was attributed solely to a continued adrenal secretion. Evidence not yet ready for publication indicates that under normal conditions, with the cervical sympathetic connections intact, the thyroid gland also may play a rôle in causing the emotional upset to continue long after the initiating experience. This extension of the visceral disturbances in time has bearings on conduct in an exciting situation, as well as thereafter. It points out the natural effect of the full expression of fear or rage, and shows the importance of limiting that expression if a persistent state of disquiet is to be avoided. It also corroborates the counsel that when bodily functions are dominated by sympathetic influences, as in the more powerful emotions, the exercise of opposed function should not be attempted. Thus the digestive processes, which are inhibited by excitement, may continue to be disturbed for a considerable period, and digestion can not proceed properly until a state of calm has been restored.

The studies on medulliadrenal secretion described in previous papers from these laboratories have been identified with the "emergency theory" of the sympathico-adrenal functions—i.e., with the view that these func-

tions are made to operate in times of stress, when they facilitate and reinforce the laboring muscles (Cannon, 1914). The involving of these functions in slight muscular contractions might be regarded as invalidating the theory. The essentials of the theory, however, were that the changes wrought in the organism at critical moments—the shifts in the circulating blood, the hyperglycemia, the dilated bronchioles, the more rapid heart beat, the flush of adrenin—were favorable to highest efficiency in supreme muscular exertion. That all these changes may occur to some small degree, even when an animal rises from repose and moves about in a simple and ordinary manner, indicates that what takes place on a large scale in emergencies takes place on a small scale in the common behavior of the organism. We can hardly conceive that slight efforts are given a critical importance because the heart has been denervated—they appear to be too slight to justify that interpretation. That being assumed, therefore, the emergency theory would have to be altered insofar as it might imply that the sympathico-adrenal mechanism is called into action *only* at times of violent emotion. According to the evidence now in hand, the greater the emergency, as measured by intensity of excitement and struggle, the more is that mechanism utilized.

SUMMARY

The evidence for and against emotional stimulation of medulliadrenal secretion is reviewed and the desirability of securing further evidence is made clear.

The advantages of the lasting preparation of the denervated heart are pointed out: *a*, the acceleration is slight (about 10 beats per minute or fewer) if thyroid, hepatic and medulliadrenal factors are excluded; *b*, the faster heart beat which occurs when medulliadrenal secretion alone is admitted to action and is stimulated justifies the use of the heart as an indicator of an increase of that secretion; *c*, the denervated heart permits a graphic record to be made which displays the latent period, the peak of activity, and the gradual subsidence of the humoral response; *d*, the observations may be repeated again and again on the same animal under normal conditions; *e*, control tests can be made on the same animal after inactivation of the adrenals (by removal of one and denervation of the other); and *f*, the increased heart rate can reasonably be interpreted as a rough measure of the increase of adrenin in the blood, for the faster circulation which would accompany increased secretion of adrenin would be unfavorable rather than favorable to its causing the cardiac acceleration.

Minor movements of the cat with denervated heart, such as extending the legs or turning the body, were accompanied by increases of heart rate of 5 to 10 beats per minute; walking made the rate faster by 10 to 20 beats. The average increase in 27 tests was 15. No signs of emotional disturb-

ances were noted. After adrenal inactivation the same activities were accompanied by very slight acceleration or none at all—the average in 23 tests was 1 beat per minute (see table 1 and figs. 1 and 2).

Emotional excitement, such as may be aroused by bringing near the cat a barking dog, or by restraining the animal in a holder, and manifested by erection of hairs and minor movements (e.g., hissing, snarling, retraction of the ears, baring of the teeth, or restless twitching of the tail) was accompanied by a faster heart rate. The increment varied roughly between 15 and 30 beats in the presence of the barking dog and between 10 and 35 beats during restraint in the holder. The average rise in 45 tests was 22. The variations of rate corresponded with variations in other signs of emotional reaction. After inactivation of the adrenal glands repetition of the same conditions with the same animals induced only minor accelerations, or none at all, or, in some instances, a small decrease of heart rate (see table 2 and figs. 1 and 2). The average in 39 tests was 2.

Great emotional excitement *plus* vigorous activity, brought about by letting a dog bark at the cat when caged, or by causing the cat to struggle in the holder, though it lasted only a minute or less, caused the heart rate to increase, in the former condition between approximately 30 and 50 beats per minute, and in the latter between approximately 40 and 80. The average rise in 53 tests was 49. After the medulliadrenal factor had been excluded, the rise of rate was usually 8 beats or fewer, and in some tests the heart worked more slowly after the struggle than before (see table 3 and figs. 1, 2, 3 and 4). The average rise in 43 tests was 5.

After a cat has been caged and excited by a barking dog outside, the disturbance thus induced may persist for twenty or twenty-five minutes, although the animal meanwhile is resting quietly on a cushion (see table 4 and fig. 5).

In the discussion some practical inferences are drawn from the foregoing observations.

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STUDIES ON THE CONDITIONS OF ACTIVITY IN ENDOCRINE GLANDS

XXI. The Rôle of Adrenal Secretion in the Chemical Control of Body Temperature[1]

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Erection of hairs, ruffling of feathers, constriction of peripheral vessels, increase of blood sugar (Embden, Lüthje and Liefmann, 1907; Freund and Marchand, 1913) are well-known phenomena occurring when warm-blooded animals are exposed to cold. They are reactions which have been interpreted as useful in protecting the organism against a reduction of the body temperature; for the raised hair and feathers enmesh a thicker layer of air around the body, the constricted surface vessels lessen the heat loss, and the extra blood sugar provides a readily combustible material for increase of heat production. Erect hairs, pallor and a higher glycemic percentage are characteristic also of emotional excitement—i.e., in the disturbances produced by cold, and by strong emotion as well, there are signs that the sympathetic division of the autonomic system is in action. The question naturally arises, Is secretion from the adrenal medulla, which is admitted to be under sympathetic control, augmented when cold causes a discharge of sympathetic impulses? The answer to this question has interesting physiological bearings, for, as shown by earlier studies in these Laboratories by Aub, Bright and Forman (1922), and by McIver and Bright (1924), absence of medulladrenal secretion lessens the rate of metabolism, and increase of that secretion accelerates the oxidative processes. If the adrenal medulla is excited to extra activity by cold the extra adrenin discharged would effect a faster burning in the body just when there would be need for it.

Cramer (1919) has published figures showing the appearance of adrenal glands taken from mice which had been exposed to extreme or to moderate cold. After exposure to extreme cold the medullary cells lose their outlines

[1] A report of our first observations was made to the National Academy of Sciences in April, 1924 (see Proc. Natl. Acad., 1924, x, 245).

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and the "adrenalin granules," which normally fill the cells as an evenly distributed fine dust, are no longer visible. After exposure to more moderate cold the appearance may be indistinguishable from the resting gland. Vincent (1925) has reported similar observations on white rats; considerable reduction of the temperature of the surroundings caused a lessening of the chromaphil reaction if the animal's temperature became lowered. In 1921 Fujii announced that the content of chromaphil substance in the adrenal medulla was reduced in rabbits which were fastened to an animal board, but that the reduction was very slight if the rabbits were protected against loss of body temperature, and did not occur at all if the splanchnic nerves had previously been cut. From these results the inference might be drawn that by rousing splanchnic impulses cold induces so great a discharge of adrenin that there is actually a depletion of the stored material in the gland. But as Trendelenburg (1923) has noted, this observation should be interpreted cautiously, for in the cooled animal the production of adrenin may be retarded or diminished. This is a comment which applies also to Cramer's and to Vincent's results.

More direct evidence of increased adrenal secretion in consequence of heat loss was furnished by Hartman, McCordock and Loder (1923) and by Hartman and Hartman (1923). They observed that when cats with a completely denervated iris are dipped in cold water, or when they are dipped in warm water and while wet are placed in a cold room or exposed to a current of cool air, the pupil is dilated if the adrenal glands are present, but not if the glands have been removed or if one has been removed and the other denervated. Their method required holding the cats firmly during the immersion, and they mention struggling by the animal as an accompaniment of the procedure. They conclude, however, that in most instances excitement did not appear to be an important factor in the reaction to cold, for no change in the pupil or only a slight change occurred when the animals were put into water at body temperature. The increased medulliadrenal secretion, which they inferred from the dilated pupil, they attributed to peripheral stimulation rather than to a general fall of body temperature.

The method used by Hartman and his associates has been severely criticized by Stewart and Rogoff (1923). They state, "A progressive dilatation of the pupil as an experiment goes on is a very common thing indeed. This has been abundantly proved in our own work to be no indication of increasing epinephrin output." The casual reader might suppose from this statement that Stewart and Rogoff had used the denervated iris. The preparation which they have employed, however, was the iris deprived only of its *sympathetic* innervation. In 1915 and again in 1919 it was pointed out that calling such a preparation a "denervated eye" was unwarranted, and that the dilatation of the pupil under the

circumstances might be complicated, e.g., it could be accounted for by central inhibition of the still active constrictor muscles of the iris, as well as by increased secretion of adrenin (Cannon, 1919). So long as two factors may be producing the same effect a sound inference is impossible until the part played by each is clearly distinguished and evaluated. Stewart and Rogoff (1920) responded that they were quite well "aware that the oculomotor nerve supplies the eye," and that they had used the expression "denervated eye" for convenience. Hartman, however, was not using a similar expression for convenience. He was doing what he said, i.e., employing the *completely* denervated iris, after removing both the ciliary and the superior cervical ganglia. Since Stewart and Rogoff have not observed the denervated iris in any exact sense, their experience has little pertinence. When they state (1923) "in our opinion" the method is quite inadequate, and "we do not think" any of Hartman's statements that cold increases the adrenin output could be proved true by his method, it is well to recognize how slight is the basis for their judgments. And when they declare that conclusions based on the denervated iris and the "denervated heart, without quantitative estimations of the output, have no real foundation," it is well to recall that the denervated heart has been used for quantitative assays of the adrenin output (Cannon and Rapport, 1921; Anrep and Daly, 1925) and that these assays showed, contrary to the assertions of Stewart and Rogoff, that the output is greatly increased by reflex stimulation and asphyxia.

Ever since the observations of Aub, Bright and Forman we have planned experiments to test the important question of a possible rôle of increased medulliadrenal secretion in the chemical control of body temperature. The results obtained by Hartman and his co-workers marked definite progress in answering that question. Because those results have been doubted, however, and also because we desired to eliminate or to minimize quite certainly the disturbing factors of emotion and struggle in the reaction to cold, we have investigated the reaction by other methods.

THE INCREASED OUTPUT OF ADRENIN DUE TO COLD. The indicator for increased medulliadrenal secretion which we have employed is the denervated heart in unanesthetized animals. In a previous paper of this series (Cannon, Lewis and Britton, 1926) the method of isolating the heart from the central nervous system and still leaving one vagus nerve to care for the alimentary tract has been described. The animals (cats) thus prepared live quite normally in the quiet conditions of the laboratory. The heart is influenced only by the effective agents brought to it in the blood stream, and by temperature changes. It is highly sensitive to circulating adrenin; Cannon and Rapport (1921) noted that intravenous injection of adrenalin, 0.00068 mgm. per kilo per minute, increased the pulse in the cat as much as 34 beats per minute; and Anrep and Daly

(1925) have reported that the isolated heart of the cat is sensitive to adrenalin in the solution 1:1,400,000,000. When one adrenal gland has been removed and the other thoroughly denervated, and the hepatic nerves have been severed, the denervated heart no longer accelerates to a noteworthy degree under conditions which normally would excite medulliadrenal secretion (Cannon, Lewis and Britton, 1926). It is, therefore, a reliable indicator.

For the purpose of the present enquiry the denervated heart has advantages over other denervated organs. First, it renders the conditions of the experiment unfavorable to the expected result; for the natural influence of cooling is to decrease the rate of the isolated heart (Knowlton and Starling, 1912), and if cooling evokes a greater output of adrenin and thereby a cardiac acceleration, the acceleration is induced in spite of an influence directly opposed to its occurrence. Again, the denervated heart has advantages in that the changes of rate whereby it manifests its response can readily be determined by auscultation or palpation, or by combination of the two, or can be easily registered by a mechanical device or in an electrocardiogram.

Method. We employed several different methods of exposing animals to cold. Our first method was that of holding the animal, warm and comfortable, in the lap near a window, and after the heart rate had been counted or registered, opening the window to let in cold air. In some respects this method was quite satisfactory—excitement and motion were eliminated because the surroundings were familiar to the animal; and the observer could listen to and record the heart beat, watch and feel for muscular quivering, and note the animal's temperature, without inconvenience. The defect of the method lay in its limitation to periods of cold weather.

We also used a refrigerating room, large enough to receive two observers, that was kept at about 5°. The disadvantages of this method of test were *a*, strangeness of surroundings which, according to our experience, may be accompanied by movement (exploration), and this by a heart beat somewhat more rapid than in the quiet state; *b*, inconvenience for continued observation, and *c*, remoteness from recording apparatus. Its great advantage, when the animal remained quiet, was complete freedom from disturbance due to excitement or struggle. Though the pulse could not be mechanically recorded, it could be easily counted by means of a stethoscope.

The most generally serviceable method which we employed was that of introducing into the stomach a known amount of cold water. Various observers have studied the influence of ingested water on metabolism. Laschtschenko (1898) observed no noteworthy increase in carbon-dioxide production when the temperature of the water was 37°. Speck (1892)

concluded from two tests on himself that metabolism is not influenced by drinking large amounts of water (1250 to 1500 cc.) but he did not report its temperature. Benedict and Carpenter (1918) performed five calorimeter experiments on subjects who, during the period of observation, drank between 1584 and 3985 cc. of water at approximately room temperature, and six respiration experiments on subjects who drank between 200 and 325 cc. of water at temperatures ranging from 50.5° to 58°, and, in one case, 500 cc. at 11°. In all the experiments except two the heat increment over the basal value did not exceed 4 per cent; in the two the increments were 15 and 16 per cent. A subject in one of these two tests (J. J. C., "an unsatisfactory subject in many ways") whose metabolism increased 15 per cent when he drank 1800 cc. at 21°, had an crease of only 3 per cent when in another test he drank 1547 cc. at 21°. It seems probable, therefore, that disturbing factors entered into these two anomalous cases. However that may have been Benedict and Carpenter concluded that when not more than 500 cc. of water are taken, whether at 22° or 55°, no significant increase of basal metabolism results. The general testimony of observers of human conditions is that drinking moderate amounts of water at intervals during a test, and with temperatures not far removed from that of the body, does not influence the metabolic rate to a noteworthy degree.

The most definite evidence that cold water increases basal metabolism is that presented by Lusk (1915). He found that when glucose was given in cold water (16.5° to 18°), or when cold water was given alone, there was a discrepancy between the results obtained by direct and indirect calorimetry—a discrepancy which disappeared when water at body temperature was used. That the discrepancy was chiefly due to extra heat production and not to passage of heat from the animal to the cold water and therefore not to the calorimeter is indicated by close correspondence of the average figures for the caloric output as determined by direct calorimetry whether the water was cold (75.19 calories in three hours) or warm (76.39 calories), and the lack of such correspondence when the determination was indirect (80.33 calories with cold water, and 75.92 with warm water).

In none of the researches cited above was cold water employed in order to study systematically the responses and adjustments which the organism might employ in order to prevent the lowering of body temperature. In our experiments we have used cold water at a fairly uniform low temperature; it was introduced into the stomach quickly by stomach tube or more commonly, in man, by rapid drinking. In the tests made on cats the animals were placed in a box provided with a sliding cover which had an opening at one end through which the head emerged. A catheter was readily introduced by passing it through a hole in a piece

of wood held between the animal's teeth by pressure with the hand on the upper and lower jaws. When in the tests on man the stomach tube was used it was gently pushed down the esophagus to the accompaniment of swallowing motions. Doubtless there was a disturbance due to the introduction of the tube, in both cat and man, but as control observations, to be reported later, have shown, it soon subsided. In the human experiments in which cold water was drunk it was siphoned through a short tube from a beaker. Thus the position of the subject was not changed and large muscles were not brought into action. Crushed ice was given by spoon in some cases, for the extra calories required to melt the ice (80 calories per gram) are added to those required to bring the melted ice to body temperature, and thereby the same task can be set the organism in smaller bulk than if water alone is employed. This idea was suggested to us by Boothby. The use of cold water has several advantages: *a*, it can be given anywhere and consequently strange surroundings are avoided; *b*, it permits direct observation of effects on body temperature, heart rate and shivering, and also ready registration of changes by mechanical or electrical methods; *c*, it is easily available at any time, and *d*, it is satisfactorily quantitative. The last advantage is most important. The weight of the animal and its rectal temperature and specific heat, the volume of the introduced water and its temperature and specific heat, can all be known. By assuming that the circulation makes the introduction of cold water into the stomach and intestines equivalent to mixing it with the body fluids (i.e., equivalent to adding the same amount of cold water to a bulk of fluid having the temperature, volume and specific heat of the organism), it is possible to calculate how much a certain amount of cold water would lower the body temperature, if no extra heat were produced or conserved. For example, if in the case described in the protocol on page 480 we should assume that the circulating blood brings heat from all parts of the body to the splanchnic region where the cold water lies exposed to an extensive capillary area, if we should assume further that the water (120 cc., at 1°, and with specific heat 1) produces only physical effects in the body of the animal (2400 grams, at 39.6°, and with specific heat 0.83), and that additional loss of the body heat to the surroundings may be neglected, the resultant temperature, after complete mixing, would be 37.3°. In other words, the temperature of the animal would fall 2.3°. The fall within five minutes was 1.8°, but after twenty minutes it was only 1.2°. Heat producing or heat conserving factors, or both, of a physiological character, must have been set at work, therefore, not only to prevent the temperature from dropping but also to restore it. The heat-producing task confronting the organism, if normal temperature is to be maintained, can thus be fairly accurately known. This may be called the "heat tax," or "heat levy," or "heat

debit," or "heat liability," or "heat debt" of the organism. We shall use the last mentioned term.[4]

A number of conditions may influence the reaction of the body to the cold water that is introduced. If the water should remain as a mass in the stomach, heat would pass into it more slowly than if the water should be pressed out from the stomach and spread along the small intestine so as to present an extensive surface. Quite possibly the rate of the gastric discharge of water into the intestine may vary somewhat in different animals, but as a rule water is quickly moved onward (see Cannon, 1911). There is little reason for supposing that an important factor lies there. Doubtless, also, the adrenin set free during the disturbance caused by passing the stomach tube may play a part in meeting the heat debt—a small part, probably, because the disturbance is brief. Again, the external temperature is undoubtedly important as affecting the influence of the "heat debt." If the room air is 19° the organism is losing more heat to the surroundings than if the room air is 25°. A given heat debt produces greater physiological effects in the former than in the latter case (see table 7). In the winter it is possible to minimize this factor by keeping the room air about 20°. Finally, the initial temperature of the water is significant; thus, though 100 cc. of water at 1° present the same heat debt as 132 cc. at 10°, when the body temperature is 38°, yet in the body the heat passes so much more rapidly into the colder than into the warmer water that these two equal "debts" would have different physiological effects (see fig. 3). In order to lessen the influence of the initial temperature factor we have generally used water between 0° and 1°. With this factor and the external temperature fairly constant, it is clear that the heat debt can be varied at will, and adjusted nicely to the weight or to the estimated surface of the animal and to the purposes of the experiment.

We did not make numerous tests on the effect of cold because while engaged in this research we were using the same animals to study the influence of emotion (see Cannon and Britton, 1926) and were so well acquainted with them and so confident concerning the significance and reliability of their responses that relatively few experiments sufficed to

[4] In the preliminary report (Proc. Natl. Acad., 1924, x, 245) on the experiments here described in detail the term "heat liability" was employed. Since then (Proc. Roy. Soc., 1924, xcvi, 442) Hill, Long and Lupton have introduced the conception that the organism by developing lactic acid beyond the oxygen requirement incurs an "oxygen debt," which must later be met by increased oxygen supply until normal conditions prevail again. The condition is similar to that which is present when cold water is introduced into the body. Since the term "*debt*" is briefer than "liability" and has already become established in physiological literature, we are using it instead of "liability."

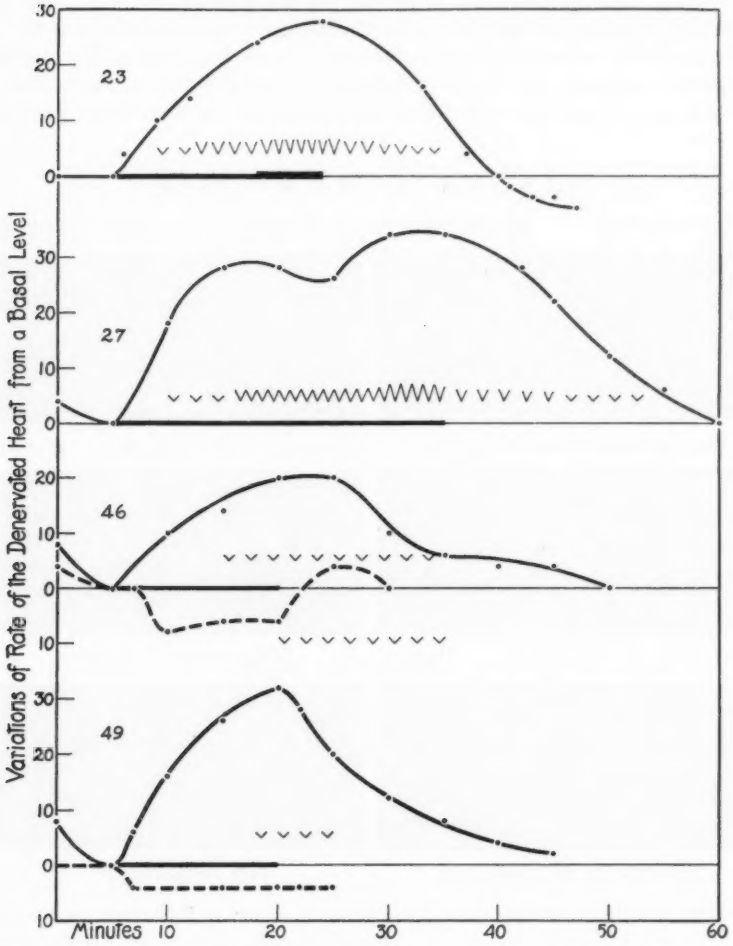

Fig. 1. Increases of rate of the denervated heart above a basal level when animals with adrenals intact (nos. 23, 27, 46 and 49) were exposed to cold air. The period of exposure is denoted by a thickening of the base line; the double thickening in the case of no. 23 marks the time of a cold draught through an open door. Shivering is indicated by separate *v*'s when it was intermittent, and by connected *v*'s when it was continuous. The size of the *v*'s marks roughly the intensity. The dash-lines in cases 46 and 49 show the changes of heart rate under similar conditions of exposure after exclusion of medulliadrenal secretion.

yield the required evidence. Also the results proved at once to be so uniformly consistent that it was not necessary to multiply them.

The effect of exposure to a cold environment. In figure 1 (no. 23) and in figure 2 are shown the effects on the rate of the denervated heart of exposing an animal, which was resting quietly, to cold air. Both adrenal

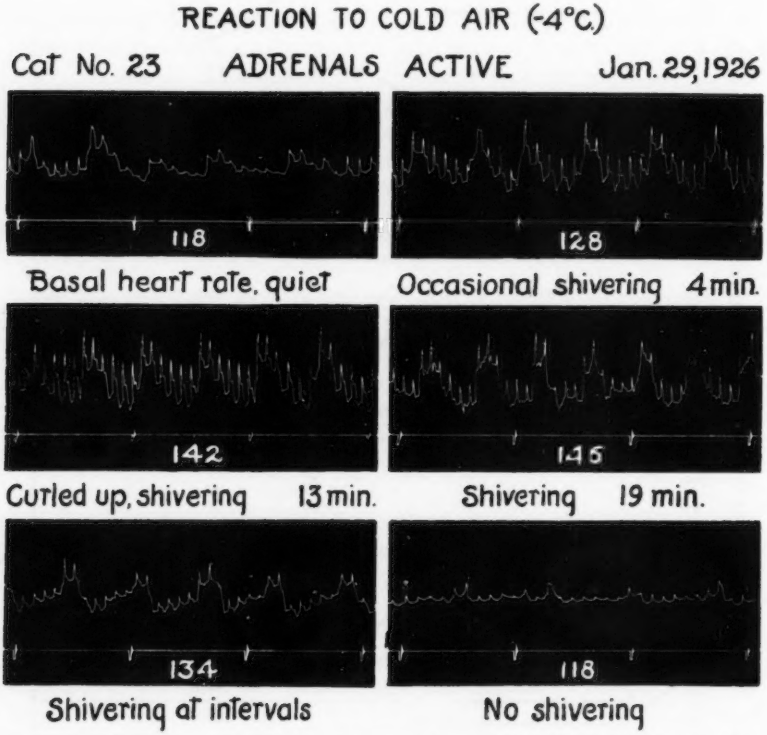

Fig. 2. Original records showing increases of rate of the denervated heart when cat 23 was exposed to air at −4°, January 29, 1926. Below the records taken while the window was open are written figures telling the time after the opening. Eight minutes after closing the window the rate had fallen from 146 to 134, and twelve minutes later to 118 (the basal). Time in five-second intervals.

glands were intact. The date was January 29, 1926. The room temperature was 16°, the outdoor temperature was −4°. The assistant sat on a table next a closed window, with the cat on a board in his lap. The tube of the recording apparatus reached to the kymograph. The basal rate was registered at 118 beats per minute. Four minutes after the win-

dow was opened the heart rate had risen 10 beats per minute. In thirteen minutes the rate had increased 24 beats. At that time the door was opened so that a strong draught of cold air rushed by. At the end of the next six minutes (i.e., 19 minutes from the start) the rate had reached 146 beats per minute—i.e., 28 above the basal, a rise of 24 per cent. The window was then closed. Nine minutes thereafter the rate had fallen from 146 to 134—16 beats above the basal; it continued to fall until in sixteen minutes after the window was closed it reached the basal (118). Seven minutes later it was 6 beats below that level. We would stress the fact that the animal throughout the test was resting quietly, so that the reaction was not complicated by stimulation of the adrenal medulla by excitement or gross bodily movements. The back was hunched up, with the feet nestled underneath the body, and the tail held close at the side. Four minutes after the window was opened the hairs were standing and continued thus until four minutes after closing the window when the animal was covered. Shivering occurred after the first four minutes of exposure; at first it was slight, limited to the shoulders, and occasional. As time passed, and coincident with the increasing heart rate, the shivering became more general, more vigorous, and was marked with each inspiration. Twelve minutes after the window was closed there was still occasional very slight shivering. This was three minutes before the heart beat returned to the basal rate.

On February 11 the adrenals of this cat were inactivated. On February 23, when the animal was again energetic, and the out-door temperature was again low (−6°), the test was repeated, with the exception that after twelve minutes near the opened window the cat was placed in a small wire cage and set outside on the ledge for ten minutes. The visible reactions of the animal to the cold were similar to those seen on January 29. The heart rate, however, instead of increasing 28 beats per minute, rose only 6 beats, and later fell 4 beats below the basal before restoration to its former condition. The only difference to account for this change in the cardiac response lay in the absence of medulliadrenal secretion. The completeness of the removal of that influence on the heart was repeatedly proved in this case by absence of the fast rate after struggle (see Cannon and Britton, 1926, table 3).

The observations on cats 27, 46 and 49 (fig. 1) were made in a refrigerating room. The rate of the heart was not mechanically recorded, but was counted by the aid of a stethoscope and stop watch. As indicated by the heavy portion of the basal line in the graph for no. 27 (fig. 1), the animal was exposed to cold for 30 minutes. During this time it rested on a metal tray supported on the observer's lap. During the first five minutes there was no shivering; the hair was slightly ruffled; the pulse rose 18 beats. During the second five-minute period the fur became very

TABLE 1

Increases of rate of the denervated heart after introducing cold water into stomach. The italicized figures were recorded in control experiments, either with warm water while the adrenals were intact or with cold water after adrenal inactivation

CAT NUMBER, DATE	CAT WEIGHT	ROOM TEMPERATURE	AMOUNT WATER GIVEN	TEMPERATURE OF WATER	HEAT DEBT* CAL. PER KILO	BASAL BEFORE WATER GIVEN	HEART RATE IN BEATS PER MINUTE											
							Increases over basal after water given											
							1–2 m.	5 m.	10 m.	15m.	20m.	25m.	30m.	35m.	40m.	45m.	50m.	55m.	60m.
	kgm.	*°C.*	*cc.*	*°C.*														
(26)																		
3/16/26	2.8	24.0	200	10	2000	120	132	64	20	12	8	6	0†					
3/20/26	2.4	24.5	130	1	2000	92	116	50	24	24	20	16	8	4	6			
3/22/26	2.4	26.5	130	34	220	90	*124*‡	*70*	*22*	*8*	*2*							
3/22/26§	2.4	26.5				90	*76*	*26*	— *6*									
4/ 1/26	2.5	21.0	135	10	1510	104	*−12*	*12*	*−12*	*−16*	*−22*	*−14*	*−18*	*−16*	*−16*	*−14*	*−16*	*−16*	*−8*
(27)																		
3/13/26	3.3		236	10	2000	130	56	44	32	20	14	12	6	2	0			
3/17/26	3.3	24.3	162	1	1820	120	44	36	32	34	26	30	20	20	12	8	4	4	0
3/22/26	3.2	26.5	162	33	250	124	*36*	*8*	*0*										
4/10/26	2.4	24.0	130	1	2000	104	*8*	*2*	*0*										
(33)																		
4/16/26	2.4	20.5	120	1	1850	118	42	34	30	22	26	26	26	18	22	22	18	16	12
4/28/26	2.7	23.0	146	1	2000	120	32	38	28	20	16	12	10	14	10	8	8	0	
4/29/26	2.6	22.0	146	31	390	132	*36*	*8*	*4*	*0*									
5/ 5/26	2.2	25.6	116	1	1950	122	*10*	*6*	*2*‖										
(46)																		
6/23/26	2.9	23.7	162	1	2070	126	22	18	18	14	12	6	2	2	0				
7/23/26	2.9	22.5	162	1	2070	120	*−8*	*−8*	*−8*	*−12*	*−10*	*−8*	*−8*	*−6*	*−4*	*0*			

(4)																		
12/22/25	1.8		73	1	1500	106	−2	2	2	4	4							
12/31/25	1.9		103	1	2000	118	0	2	2	2	2							
(92)																		
12/22/25	2.2		89	1	1500	140	2	−8	−8	−12	−12							
(95)																		
1/ 2/26	2.95		160	10	1520	164	−4	−12	−8	−10	−16	−18	−8					
(110)																		
12/22/25	2.05		73	1	1320	126	4	0	4	0	4							

* The extra heat which the organism must produce or conserve in order to maintain its own temperature—the "heat debt" —is calculated from the amount of water injected and the need for raising the temperature of that water from 1° or 10° to 38°C.

† No shivering during this test.

‡ Considerable struggle.

§ Stomach tube passed only.

‖ Cat vomited the water.

rough, there were occasional slight tremors, and the pulse rate increased 10 beats more, i.e., to 28. Then, as shown in the graph, shivering became general and continuous, though moderate, with no marked change in the cardiac contractions, until ten minutes had passed. By this time the hair was bristling on all exposed parts of the body. Thereupon the shivering became gradually more and more vigorous, and the animal became restless. The heart rate rose to 34 beats above the basal figure, a rise of 27 per cent. At the first observation made after removing the animal from the cold room the rate had fallen 6 beats; it continued to fall gradually until at the end of twenty-five minutes in warm surroundings it reached the basal again. No control tests for the effects of cold were made on this animal after adrenal inactivation; but the repeated failure of the heart to accelerate in the supreme test of struggle, after exclusion of medulliadrenal influences (see Cannon and Britton, 1926, table 3), proved that the acceleration in the cold room resulted from increased output of adrenin.

The graph for no. 46, figure 1, is similar to that for no. 27, and is self-explanatory. The percentage increase of heart rate was 18. When, after the right adrenal gland had been excised and the left denervated, the animal was again kept in the cold room fifteen minutes, as before, the heart rate, instead of rising, fell 8 beats per minute. It continued low until the animal was brought, shivering, into a warm atmosphere. There followed a quick rise to 4 beats above and then a subsidence to the basal.

The protocol of observations on no. 49 (fig. 1) in a cold-room experiment is given below. Taking the rectal temperature of the cat would be so disturbing that it would vitiate the experiment. Instead, the temperature was taken by holding the thermometer enclosed in the inguinal fossa.

Cat 49. June 22, 1926. In hallway, temp. 23.5°. Inguinal temp. of cat 38.7°. 11:40, basal h. r. (heart rate), 104 beats per minute. 11:45, h. r. 96. 11:46, *in cold room* on metal tray; temp. room 5.5°. 11:47, h. r. 104. 11:50, cat huddled up, hairs rough on back; h. r. 112. 11:55, no shivering, slight restlessness (movement of feet), inguinal temp. 37.9°, h. r. 122. 11:58, slight tremors in shoulder region, associated with inspiration. 12:00, hairs erect, inguinal temp. 37.1°, h. r. 128 (an increase of 33 per cent). 12:02, *out of cold room*, in hall, temp. 23.5°, h. r. 124. 12:05, slight tremors occasionally with inspiration, hairs smooth, inguinal temp. 37.9°, h. r. 116. 12:10, very quiet on lap, cat curled up on cushion, no tremors, h. r. 108. 12:15, inguinal temp. 38.1°, h. r. 104. 12:20, curled up, very quiet on lap, h. r. 100. 12:25, same condition, h. r. 98.

On June 24, the adrenals were inactivated in this cat. Thereafter for ten days all tests (exposure to dog and struggle) failed to cause any marked increase of heart rate. And when (July 2) the animal was taken to the cold room for 15 minutes the heart, instead of accelerating, beat more slowly (see fig. 1).

The effect of introducing cold water. In observations on the denervated heart after introducing cold water into the stomach there was found a very rapid rate immediately or as soon as the beats could conveniently be counted (see table 1 and fig. 3). This acceleration is due to the disturbance of the animal on being confined to a box and having a catheter passed through the mouth and esophagus, for it occurs for a brief period after that procedure alone (see table 1, no. 26, March 22) and also after introducing warm water (see table 1, no. 33, April 29, and fig. 3, *C*). In about ten minutes the rate falls from the initial peak to a plateau, and after remaining there for a varying time it gradually returns to the former basal value (see fig. 3, *B*).

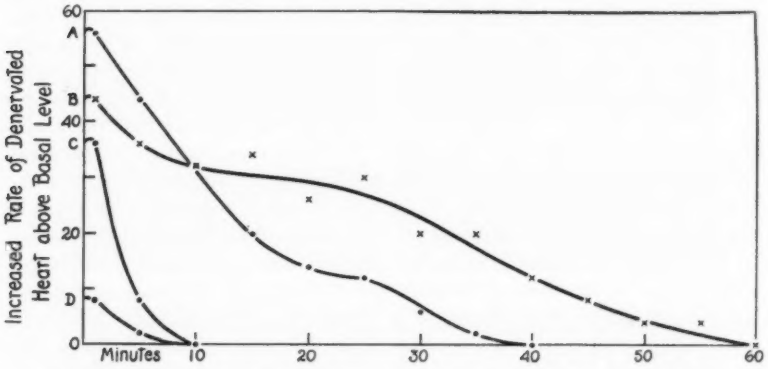

Fig. 3. Increases of rate of the denervated heart in cat 27, responding to heat debts produced by introducing water into the stomach under different conditions. *A*, *B* and *C*, with adrenals active. *A*, a heat debt of 2000 small calories when the water was given at 10°. *B*, a heat debt of 1820 calories, water at 1°. *C*, a heat debt of 250, water at 33°. *D* (with adrenals inactivated), a heat debt of 2000 calories water at 1°.

The results summarized in table 1 show the effects produced by heat debts between approximately 1500 and 2000 small calories per kilo when the temperature of the water was 10° (no. 26, March 16, and no. 27, March 13) and when the temperature was 1° or less (nos. 26, 27, 33 and 46). In the same animal the colder water had a more prolonged influence in speeding up the denervated heart than did the warmer water, though the heat debt was about the same (cf. fig. 3, *A* versus *B*). Comparison of the increments of heart rate after the establishment of the same heat debt, with the adrenal glands intact and after their inactivation, brings out a remarkable difference (cf. also *B* and *D*, fig. 3). Instead of an acceleration ranging between approximately 20 and 30 beats per minute in

the plateau region and representing (with the exception of no. 46, which on June 23 had a respiratory infection) increments varying between 23 and 28 per cent, that did not completely disappear for 40 or 60 minutes, when the adrenals were active, the acceleration, *after* the adrenals were excluded, either failed to occur (as in no. 26, April 1; and no. 46, July 2) or was slight even at the peak and usually had disappeared at the end of ten or fifteen minutes (see no. 27, April 10 (fig. 3, *D*); and no. 33, May 5).

The last five series of figures in table 1 were obtained by tests on cats with known adrenal inactivation (see Cannon, Lewis and Britton, 1926). No tests were made before the inactivation. The results closely correspond to those of the controls described in the preceding paragraph.

Another mode of control was that of comparing, when the adrenals were capable of functioning, the effect of a given volume of water at 1° with that of the same volume nearly as warm as the body (table 1). Although the heat debts in the latter instances varied between 220 and 390 small calories it is probable that they were relatively so low that the differences were not significant. Certainly the effects did not indicate that the variations had any influence. Naturally the heart beat faster at the start because of the upset of the quiet state when the water was given, but the faster beat, instead of lasting 40, 50 and 60 minutes or more, as it did in response to cold water, was recovered from in 10 or 15 minutes. Indeed in two cases the period of cardiac acceleration was only a little longer after warm water than it was after the passage of the catheter alone. The slower return of the heart to its former rate in no. 26 on March 22 may have been due to the considerable struggle in the box when the water was given.

Protocols presenting details of observations on cat 33 (see fig. 4) follow:

Cat 33, April 16, 1926. *Adrenals active.* Basal h. r. (heart rate), 118 beats per minute; inguinal temp. 39.6°, room temp. 20.5°. At 2:52 p.m., 120 cc. water at 1° (1850 cal. per kilo) given by stomach tube. At 2:56, h. r. 160, hairs standing. 2:57, h. r. 152, occasional shivering, inguinal temp. 37.8°. 3:02, h. r. 148, inguinal temp. 37.8°, fur rough. 3:07, h. r. 140, shivering more frequent. 3:12, h. r. 144, inguinal temp. 38.4°. 3:17, h. r. 144. 3:22, h. r. 144, shivering with inspiration, otherwise very quiet in lap, room temp. 20°. 3:27, h. r. 136, inguinal temp. 38.7°, occasional tremors. 3:32, h. r. 140, slight shivering. 3:38, h. r. 140, no shivering. 3:42, h. r. 136. 3:47, h. r. 134. 3:49, h. r. 132, very quiet in lap all this time. 3:52, h. r. 130.

April 29, 1926. *Adrenals active.* Basal h. r., 10:42 a.m., 132 beats per minute. At 10:50, 146 cc. *warm* water (at 31°) by stomach tube, vigorous struggling while water given. 10:51, h. r. 168. 10:52, h. r. 160. 10:55, h. r. 140. 10:58, h.r. 134. 11:00, h. r. 136. 11:05, h. r. 132.

Fig. 4. Original records showing increases of rate of the denervated heart when cat 33 with active adrenals was given, April 16, 120 cc. of water at 1° (heat debt, 1850 small calories), and when, with adrenals inactivated, it was given, May 5, 116 cc. of water at 1° (heat debt, 1950 calories). Below each record are stated the prevailing conditions and the interval since the water was given. Time in 5 seconds.

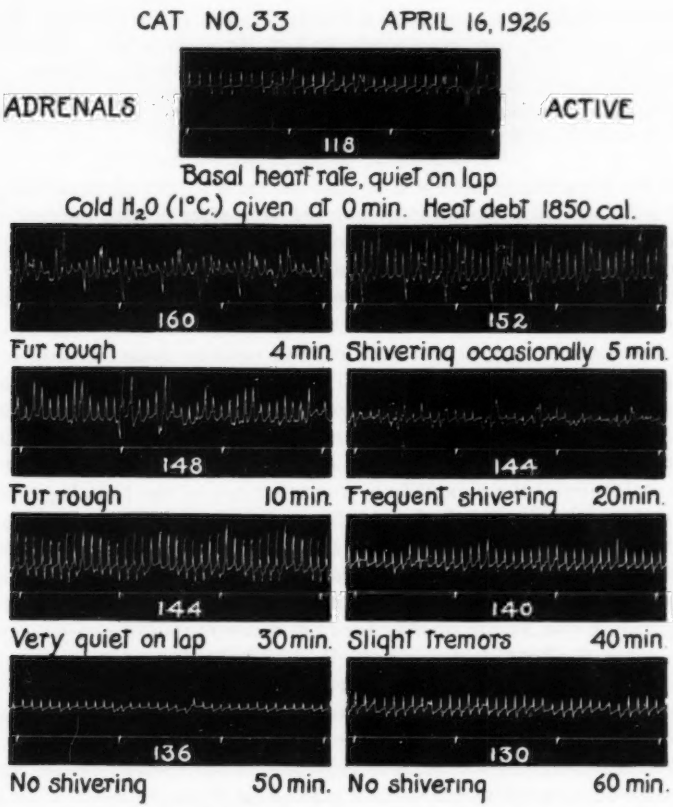
CAT NO. 33 APRIL 16, 1926
ADRENALS
ACTIVE
118
Basal heart rate, quiet on lap
Cold H_2O (1°C.) given at 0 min. Heat debt 1850 cal.
160
Fur rough 4 min.
152
Shivering occasionally 5 min.
148
Fur rough 10 min.
144
Frequent shivering 20 min.
144
Very quiet on lap 30 min.
140
Slight tremors 40 min.
136
No shivering 50 min.
130
No shivering 60 min.
CAT NO. 33 May 5, 1926
ADRENALS
INACTIVE
122
Basal heart rate, quiet on lap
Cold H_2O (1°C) given at 0 min. Heat debt 1950 cal.
128
Slight shivering 5 min.
124
Frequent shivering 10 min.

Fig. 4

May 5, 1926. *Adrenals inactive.* 3:35 p.m., basal h. r., 122 beats per minute, inguinal temp. 39.5°, room temp. 25.6°. At 3:47, 116 cc. water at 1° (1950 cal. per kilo), given by tube. 3:48, h. r. 132. 3:52, h. r. 128, moderate shivering. 3:57, h. r. 124 frequent shivering, inguinal temp. 38.2°, very restless on lap. 4:02, inguinal temp. 37.2°, cat vomited large amount of watery fluid. 4:12, inguinal temp. 37.8°. 4:17, occasional slight shivering.

In the foregoing protocols note that on April 16 the inguinal temperature fell from 39.6° to 37.8° in five minutes after a heat debt of 1850 calories was established. Calorigenic agencies were at once invoked as shown by the faster pulse and by shivering. There was no further fall. During the next fifteen minutes the inguinal temperature rose to 38.4°. The room temperature near the cat was 20.5° to 20°. After the adrenals had been inactivated, a heat debt of 1950 calories caused a drop of the inguinal temperature from 39.5° to 38.2° in ten minutes, and to 37.2° in the next five minutes. The room temperature (25.6° as compared with 20.5° before) was unfavorable to this marked lowering of the temperature of the animal. The ejection of a considerable amount of the cold water saved the animal from a continuance of the heat loss. Since the maximal increase of the heart rate was only 10 beats, and ten minutes after the water was given the rate had returned to 124, only two beats above the basal (122), the experiment had proved a satisfactory control.

It is noteworthy that when the heat debt was 2000 calories and the room temperature was 23° (in no. 33, April 28) a more rapid recovery occurred than when the heat debt was only 1850 and the room temperature was 20.5 (April 16). This effect of warm surroundings was illustrated also in experiments on no. 41, which have not been reported above because in this case vagus connections were renewed before control experiments were performed. In no. 41, a heat debt of 2000 calories (water at 1°) caused cardiac acceleration, with a plateau at 48 beats above the basal, that lasted for 65 minutes when the room temperature was 19°; the same heat debt caused an acceleration lasting only 30 minutes when the room temperature was 25°. Results of similar character were noted in human cases, to be described below.

Another condition which is doubtless important in affecting the degree of the cardiac response is the presence of shivering. In the above cases it differed in duration and intensity, as already noted (see fig. 1). It was usually most marked when the heart rate was most elevated. But it is not a necessary condition for the faster pulse—e.g., no. 26 on March 16 (table 1) met the heat debt of 2000 calories (water at 10°) with no shivering whatever. And regularly shivering does not occur at the beginning or near the end of the cardiac response (see especially nos. 46 and 49, fig. 1). In the cases reported above, however, the heat debt was paid not by the one process of increased discharge of adrenin, as indicated

solely by a more rapid heart beat, but also by muscular tremors. This muscular activity may be associated with an increased stimulation of the adrenal medulla (see Cannon and Britton, 1926), and in so far as a larger output of adrenin augments heat production in the body it may lessen the need for shivering. These points will be considered again.

All methods which we have used to threaten a fall of body temperature have evoked noteworthy acceleration of the denervated heart, so long as the adrenal glands were in their proper functional relations. When one of the glands was removed and the other denervated, exposure of the animal to cold surroundings and introduction of cold water into the stomach no longer caused the faster heart beat. The original and the control observations were made on the same animals. Repeated tests (excitement, struggle) on these animals further confirm the conclusion that adrenal inactivation alone explains the absence of rise in pulse rate. The faster rate of the denervated heart, therefore, which appears when there is danger that the body temperature will fall, is due to an increased secretion from the adrenal medulla.

THE COMPENSATORY CALORIGENIC EFFECT OF ADRENAL SECRETION EVOKED BY COLD. In a review of the literature Boothby and Sandiford (1923) have called attention to the work of various observers, beginning in 1906, who have noted that administration of adrenalin causes increased oxygen consumption or increased metabolism of isolated organs or of the body as a whole. This evidence of a pharmacological action of adrenalin on the rate of oxidation was given a physiological significance when Aub, Forman and Bright (1921, 1922) found that after removal of the adrenal glands, although blood pressure and temperature were unchanged, the metabolic rate dropped as much as 25 per cent below the previous normal level. In animals anesthetized with urethane they discovered that the standard metabolism, which fell promptly after adrenalectomy, could be restored by intravenous injection of adrenalin in approximately the amount per kilo that is produced by reflex stimulation. An increase of metabolism in man, when adrenalin was injected subcutaneously so as to be distributed in the body at about the physiological rate, was reported by Peabody and his co-workers (1918), by Tompkins, Sturgis and Wearn (1919) and by Sandiford (1920); and Boothby and Sandiford (1923) proved that intravenous injections of adrenalin in dogs, in doses which matched the secreting capacity of the adrenal medulla, notably increased the speed of heat production. Since injected adrenalin increases metabolism, and adrenalectomy decreases it, the inferences seemed justified that absence of the medulla and its secretion, rather than absence of the cortex, is responsible for slower metabolic rate after removal of the adrenals; and conversely that secreted adrenin helps to maintain the normal metabolic rate and by a larger output may increase that rate. Definite evidence

in support of the latter inference has been provided by McIver and Bright (1924), who showed that direct nervous and also reflex stimulation of adrenal secretion promptly augments metabolism to a remarkable degree —even as much as 50 per cent.

Evidence presented in the foregoing pages has shown that medulliadrenal secretion is increased when the organism is exposed to the danger of too rapid heat loss, and the testimony just cited proves that adrenin, secreted in natural amounts, is capable of accelerating in the body the heat production. Here are the factors of a physiological reaction the services of which to the organism ought to be demonstrable. We have attempted in two ways to test the capacity of these reactions: we have studied the effect of a certain heat debt on shivering, when the adrenal glands were active or inactive; and we have observed in man the influence of a heat debt on the metabolic rate in the absence and also in the presence of shivering.

The effect of a heat debt on shivering when the adrenal glands are present or absent. In discussing the cases summarized in table 1, we pointed out that animals may meet the heat debt which confronts them by combined calorigenic reactions—by a greater discharge of adrenin, as indicated by a greatly increased heart rate, and by shivering. Doubtless there are other factors which may be called into play, e.g., a saving of heat by peripheral vasoconstriction—a process the efficacy of which is much affected by the temperature and moisture of the air. For the present, however, we are considering the response of the adrenal medulla in relation to shivering. In all the animals of table 1, in which this response was examined, the heat debt was large—about 2000 calories per kilo. Since, even when shivering complicates the reaction to a threatened heat loss, increased medulliadrenal secretion occurs before the shivering begins and may well outlast it, and since the secretion may occur without any shivering whatever, and since the greater output of adrenin results in more rapid metabolism, we should be able to select a heat debt for which the increased secretion would be barely adequate without the help of shivering, and this debt in the absence of the secretion ought, theoretically, to call forth shivering. One might argue that after removal of one adrenal gland the same heat debt per kilo should not be induced as before, because even a single adrenalectomy depresses the metabolic rate (Barlow, 1924). This lowered rate, however, serves to emphasize the importance of the adrenal, in all probability the medullary portion, in the maintenance of a normal heat production. And since the object of our tests was to learn whether in the absence of adrenin the organism would resort to other factors for the heat needed to prevent a fall of temperature we felt justified in keeping the heat debt uniform. The debt which we found to be critical was 900 small calories per kilo. The figure (900 calories) was

TABLE 2

Presence or absence of shivering where there was a heat debt of about 900 calories in (A) cats with normally innervated adrenal glands, and in (B) cats with one adrenal gland removed and the other denervated

A. ADRENALS BOTH INNERVATED								B. ADRENALS, ONE OUT, THE OTHER DENERVATED							
Cat	Date	Temperature of room	Weight of cat	Amount of water	Temperature of water	Shiver	Remarks	Cat	Date	Temperature of room	Weight of cat	Amount of water	Temperature of water	Shiver	Remarks
		°C.	kgm.	cc.	°C.	min.				°C.	kgm.	cc.	°C.	min.	
116	Feb. 14	22	3.9	98	0.2	0*	Lean	263	Feb. 14	21.6	3.5	83	0.3	0	
A	Feb. 21	19.5	2.9	70	0.0	0	Med. fat	233	Feb. 18	21.8	3.4	82	0.5	15	
B	Feb. 21	19.7	3.1	75	0.1	3	Lean	110	Feb. 19	19.5	2.9	70	0.5	3	Med. fat
C	Feb. 21	19.8	3.8	92	0.2	0	Sl. fat	90	Feb. 19	19.7	3.2	78	0.1	7	Med. fat
D	Feb. 21	19.8	3.2	77	0.1	0	Lean	112	Feb. 19	19.7	4.0	98	0.1	0	Med. fat
E	Feb. 21	20	3.5	85	0.1	0	Med. fat	116	Feb. 19	19.7	3.9	97	0.2	17*	Lean
F	Feb. 21	20	3.7	90	0.2	0	Very lean	109	Feb. 19	20.4	3.3	80	0.7	6	
G	Feb. 21	20	2.8	68	0.0	3	Very lean	90	Mar. 22	21.0	3.0	73	0.2	8	Med. fat
H	Feb. 21	20.2	4.0	97	0.2	0	Med. fat	110	Mar. 22	21.0	2.7	65	0.4	3	Med. fat
244	Apr. 9	21	1.95	47	0.4	0*	Lean	110	Apr. 7	20.5	2.9	70	0.3	4	Med. fat
I	Apr. 11	20.5	2.8	68	0.7	0	Lean	112	Apr. 7	21.0	4.0	97	0.2	13	Med. fat
J	Apr. 11	21	2.6	63	0.5	0		90	Apr. 7	21.0	3.1	75	0.2	5	Med. fat
K	Apr. 11	21	3.5	85	0.5	0	Med. fat	240	Apr. 7	21.0	4.0	97	0.4	16	Lean
L	Apr. 11	21	3.2	78	0.3	0	Med. fat	244	Apr. 22	21.5	2.05	50	0.4	11*	Lean
M	Apr. 22	21.5	3.5	85	0.2	0	Med. fat	240	Apr. 22	22.0	4.0	97	0.3	16	Lean

*The same animals before and after inactivation of the adrenal glands.

reached after some preliminary tests above and below it. The animals had been fed the previous afternoon. They were observed in a routine manner after cold water was introduced into the stomach. They were placed on a table where, with hairs bristling, they usually crouched in a huddled position. A nearby thermometer showed the temperature of the room. The animals were not touched. They were carefully watched for shivering (a response which, if it occurred, was readily recognized by the quivering of the erect hairs) until the reaction passed. In different animals and at different times there was considerable variation in the degree of spread of the shivering reaction over the body.

As shown in table 2 a heat debt of 900 small calories rarely causes shivering in animals having intact adrenals—it occurred in only 2 cases out of 15. Whether the animals were lean or fat made no obvious difference in the responses. That the sympathetic system was active in these cases was indicated by the bristling hairs along the back. Under such conditions, as previously shown, the adrenal medulla is stimulated to extra secretion. Even when shivering did appear, it lasted only a short time. In the first of the two instances in section A, table 2 (B, Feb. 21), there was slight shivering of the hind legs at 10:30, that had become vigorous and spread over the whole body a minute later and that had quite passed off by 10:33; in the second instance (G, Feb. 21), a slight shivering was noted at 3:18, that had disappeared by 3:21. On the other hand, after the adrenal factor in heat production had been eliminated by removal of one gland and denervation of the other, the same heat debt caused shivering in 13 cases out of 15 observations, and furthermore, the shivering usually lasted for periods considerably longer than in the control series—for 6, 7, 8, 11, 13 and even 16 and 17 minutes instead of 3 minutes. The animal in section B, table 2, that shivered for 17 minutes on February 19 was the same animal that was recorded in section A, table 2, as not shivering at all on February 14; the adrenals were inactivated on February 16. Compare also the record for April 9 (no shivering) and that for April 22 (shivering for 11 minutes); these results were obtained on the same animal and the difference between them may be accounted for by the inactivation of the adrenal glands on April 12. In some of the tests preliminary to the selection of 900 calories as a standard heat debt the animals with intact adrenal glands met the demand for approximately 1000 calories per kilo without shivering whereas one of the animals deprived of the adrenal factor shivered for 11 minutes when confronted with a heat requirement of 840 calories per kilo.

Our previous observations have shown that when an animal has to develop heat to prevent lowering of body temperature it may rely both on the calorigenic effect of increased adrenal secretion and on the extra heat accompanying reflex muscular contractions. The results just de-

tailed indicate that the primary protection against a heat deficit is found in activities of the sympathetic system (bristling of hairs, probably vasoconstriction and hyperglycemia, and surely by increased medulliadrenal secretion), for these responses occur before the onset of shivering, and may be the only manifestations of a heat debt. If animals have been deprived of the calorigenic service of the adrenal medulla, however, greater demands are made on muscular activity as a means of maintaining the normal temperature—then, in the presence of a given need for heat shivering occurs more frequently and lasts longer. The non-use of the shivering mechanism, or the relatively slight reliance on it when the adrenals are present, implies that the secretion poured out by them when the organism is in danger of cooling is an efficient means of increasing heat production.

The influence of heat debt on the metabolic rate, independent of shivering. Since a sufficient heat debt will evoke increased adrenal secretion without attendant shivering, and since increased adrenal secretion markedly accelerates metabolism, it should be possible to demonstrate a faster metabolic rate in response to the bodily need for heat, and this quite apart from any muscular quiverings or contractions. In order to secure evidence on this point we have studied the change in the metabolic rate when a known heat debt was created by giving cold water (1° or colder) or cold water and ice. The subjects were men and women—students and workers in the laboratory—who were in vigorous health. The determinations of the metabolic rate were made in the morning before breakfast. After the subject had rested on a couch for a half-hour, two or three preliminary tests were made. One subject (A. Q., table 3) took the cold water by stomach tube. While still lying on the couch he allowed the tube to be passed; and then the water, which had been measured previously and which was kept cold in a flask set in an ice-salt mixture, was quickly poured into a funnel at the top of the tube. As soon as the water had run out of the funnel the tube was withdrawn. At first there was considerable disturbance due to this procedure but after a few trials the subject became accustomed to it and the difficulty largely disappeared. In other determinations it was found that the water could be taken fairly quickly by being siphoned through a tube reaching from the elevated flask to the subject's lips. In some instances the heat debt due to the cold water was increased by swallowing small lumps of ice which were placed in the subject's mouth from a spoon (see table 3). Usually three or four minutes elapsed after the water or water and ice were administered before the series of determinations was started. Thereafter the subjects did not complain of any discomfort, though in three instances there was testimony of a sensation as of a cold wave sweeping over the back, associated with the phenomenon of "goose flesh," i.e., the equivalent of erection of hairs and indicating activity of the sympathetic system. In three of the thirty-

TABLE 3

Records of the increase of the metabolic rate (without shivering) when a heat debt was established by cold water taken into the stomach, and of the absence of such increase when similar amounts of warm water were taken (italicized figures). Calculations based on general body temperature of 36° (see axillary temperatures, tables 4, 5 and 6)

DATE	SUBJECT	SEX	AGE	WEIGHT	HEIGHT	TEMPERATURE OF ROOM	AMOUNT OF		HEAT DEBT	METABOLISM CAL./SQ.M./HR.		TIME OF MAXIMUM METABOLISM AFTER H_2O	INCREASE IN METABOLISM	REMARKS
							Ice	H_2O		Preliminary	Maximum			
				kgm.	*mm.*	*°C.*	*grams*	*cc.*	*cal./kgm.*			*min.*	*per cent*	
1/22/25	S. W. B.	♂	32	73.0	177.0	14.4	143	520	476	41.1	53.0	17	28.5	Very low room temperature. Shivering later
2/ 4/25	S. W. B.					21.0	150	375	418	38.5	42.7	27	11.0	
5/25/25	S. W. B.					*19.5*		*600*	*98*	*39.5*	*41.8*	*7*	*5.8*	*Control. 600 cc. H_2O at 25.0°C.*
10/25/24	D. E. S. B.	♂	23	70.0	182.0	20.0	150	482	496	36.3	46.4	20	27.7	
11/11/24	D. E. S. B.					22.7	153	463	492	37.8	43.6	31	15.4	
2/ 2/25	D. E. S. B.					20.0	148	391	435	38.8	42.7	23	10.0	
4/ 5/25	D. E. S. B.					22.7	140	543	472	38.7	44.6	30	15.2	
7/17/25	D. E. S. B.					*26.5*		*557*	*47*	*35.7*	*37.8*	*6*	*6.0*	*Control. 557 cc. H_2O at 30.0°C.*
5/13/25	R. W. D.	♂	22	65.8	171.0	21.8	142	346	444	38.1	49.4	18	29.8	
6/11/25	R. W. D.					*23.5*		*490*	*45*	*41.1*	*41.4*		*0.0*	*Control. 490 cc. H_2O at 30.0°C.*
6/20/25	A. J. E.	♀	29	60.0	168.7	*24.5*		*420*	*42*	*32.0*	*33.7*	*7*	*5.0*	*Control. 420 cc. H_2O at 30.2°C.*
6/20/25	A. J. E.					25.0	139	260	424	30.4	34.6	28	14.0	Immediately following control
4/15/25	J. L. H.	♀	25	68.8	164.5	20.5	120	400	384	30.6	34.0	24	11.0	
5/26/25	J. L. H.			68.0		*19.5*		*520*	*46*	*35.0*	*36.4*	*8*	*4.0*	*Control. 520 cc. H_2O at*

4/15/25	J. L. H.	♀	25	68.8	164.5	20.5	120	400	384	30.6	34.0	24	11.0	
5/26/25	J. L. H.			68.0		*19.5*		*520*	*46*	*35.0*	*36.4*	*8*	*4.0*	*Control. 520 cc. H_2O at 30.0°C.*
5/26/25	J. L. H.					20.0	130	354	409	35.1	39.9	27	13.7	Very slight shivering previous to the period used in calculating the maximum rise
8/ 4/25	J. T. L.	♂	27	66.5	166.5	*25.5*		*500*	*45*	*34.7*	*35.2*	*7*	*1.0*	*Control. 500 cc. H_2O at 30.0°C.*
9/28/25	J. R. L.	♂	26	72.0	177.5	*22.8*		*650*	*49*	*37.7*	*39.2*	*11*	*3.4*	*Control. 650 cc. H_2O at 30.5°C.*
9/28/25	J. R. L.					24.5	138	335	389	37.6	43.2	10	15.0	
10/23/24	R. R. L.	♂	24	74.0	179.2	18.4		740	350	39.2	43.1	32	10.0	
11/ 4/24	R. R. L.					20.5	148	576	512	40.0	43.4	25	8.4	
2/19/25	R. R. L.					19.0	148	337	385	39.6	43.9	25	11.0	
6/27/25	R. R. L.					*23.0*		*600*	*49*	*40.1*	*39.6*	*8*	*−1.3*	*Steady drop in metabolism after 39.6*
6/ 4/25	J. D. P.	♂	22	59.0	169.0	28.0	145	595	527	38.6	41.9	24	8.5	High room temperature
4/14/25	J. D. P.					21.0	130	430	485	40.4	55.7	14	38.0	"No shivering." Subject very quiet
5/27/25	J. D. P.					*20.0*		*623*	*63*	*38.9*	*40.7*	*14*	*4.8*	*Control. 623 cc. H_2O at 30.0°C.*
1/16/24	A. Q.	♂	23	54.2	156.0	21.0		750	471	37.1	42.7	21	15.0	
1/22/24	A. Q.					18.5		665	441	37.8	45.8	34	21.0	Slight shiver 14 minutes after water
1/25/24	A. Q.					21.2		750	484	37.9	43.1	23	14.0	
2/ 5/24	A. Q.					24.0		750	498	36.8	46.3	16	25.0	
2/ 2/24	A. Q.					*22.5*		*750*	*21*	*41.5*	*43.8*	*11*	*5.4*	*Control. 750 cc. H_2O at 34.5°C.*
10/22/24	A. T.	♀	49	67.2	171.3	20.0	150	450	483	35.5	40.3	26	13.5	
3/12/25	A. T.			66.0		21.5 19.0	117	355	383	33.6	38.2	32	13.6	
7/28/25	A. T.					*24.0*		*600*	*58*	*31.3*	*31.5*	*9*	*0.5*	*Control. 600 cc. H_2O at 29.6°C.*

TABLE 4

Complete records of two subjects in whom a heat debt was met by increased metabolism without shivering. The figures for the control records are italicized. The axillary temperatures are in parentheses

DATE	SUBJECT	TEMPERATURE OF ROOM	HEAT DEBT	METABOLISM—CAL. PER SQ. M. PER HOUR								TIME OF MAXIMUM METABOLISM AFTER H_2O	INCREASE IN METABOLISM
				Preliminary	I	II	III	IV	V	VI	VII		
		°C.	cal./kgm.									min.	per cent
10/23/24	R. R.L.	18.4	350	39.2	41.5	42.1	41.9	43.1	41.1	42.5	39.7	32	10.0
				(36.3)	*(35.4)*	*(35.6)*	*(35.8)*	*(35.8)*	*(35.8)*	*(35.9)*	*(36.0)*		
11/ 4/24	R. R.L.	20.5	512	40.0	41.3	40.8	43.4	41.9	41.6	40.4	40.1	25	8.4
				(36.4)	*(36.0)*	*(35.9)*	*(35.9)*	*(36.0)*	*(36.0)*	*(36.0)*	*(36.1)*		
2/19/25	R. R. L.	19.0	385	39.6	40.8	42.4	43.9	42.3	43.2	41.0	41.3	25	11.0
				(36.2)	*(36.0)*	*(35.8)*	*(35.7)*	*(35.6)*	*(35.7)*	*(35.7)*	*(35.7)*		
*6/27/25**	R. R. L.	*23.0*	*49*	*40.1*	*39.6*	*39.6*	*38.6*	*37.2*				*8*	*−1.3*
				(36 6)	*(36.6)*	*(36.6)*	*(36 6)*	*(36.6)*					
10/25/24	D. E. S. B.	20.0	496	36.3	43.5	46.4	42.3	42.3	41.5	39.5		20	27.7
				(36.3)	*(35.1)*	*(34.7)*	*(34.8)*	*(35.2)*	*(35.4)*	*(35.5)*			
11/11/24	D. E. S. B.	22.7	492	37.8	39.5	41.1	39.5	43.6	41.3			31	15.4
				(36.4)	*(35.8)*	*(35.9)*	*(35.9)*	*(36.0)*	*(36.0)*				
2/ 2/25	D. E. S. B.	20.0	435	38.8	39.7	39.9	42.7	39.7	39.6			23	10.0
				(36.4)	*(35.6)*	*(35.5)*	*(35.4)*	*(35.3)*	*(35.2)*				
4/ 5/25	D. E. S. B.	22.7	472	38.7	40.3	40.0	41.5	44.6	38.5	40.9	40.0	30	15.2
				(36.4)	*(36.0)*	*(36.0)*	*(36.1)*	*(36.0)*	*(36.0)*	*(36.1)*	*(36.2)*		
7/17/25†	D. E. S. B.	*26.5*	*47*	*35.7*	*37.8*	*37.8*	*36.5*					*6*	*6.0*
				(36.3)	*(36.3)*	*(36.3)*	*(36.3)*						

* *Control. 600 cc. water at 30.2°C.*

† *Control. 557 cc. water at 30.0°C.*

three determinations reported in table 3 (S. W. B., Jan. 22; J. L. H., May 26; and A. Q., Jan. 22) there was slight shivering, but not during the period used for comparison with the preliminary basal period. The body temperature was taken in the axilla because the mouthpiece excluded use of the buccal region and because cold water in the abdomen might produce a local temperature change in the rectal region. The chief defect of the method was an onset of restlessness 50 or 60 minutes after the water was given, due to tension of the urinary bladder. Since the maximal changes of metabolism occurred well within this time, however, the restlessness did not disturb the essential conditions of the experiment.

The metabolism was measured by oxygen consumption alone. The Benedict apparatus as modified by Roth (1923) was used to determine the rate of oxygen utilization. The record of the movement of the oxygen cylinder was written on a smoked drum. As recommended by Roth, the single periods lasted a little longer than six minutes. The metabolism of the subject, in calories per square meter per hour, was calculated by the Du Bois formula.

It should be clearly understood that these experiments on human subjects were in all respects equivalent to those on lower animals in which the heat debt, without evoking shivering, augmented the output of adrenin—a calorigenic agent.

In table 3 are presented the main data of 33 tests on 11 subjects. The detailed data of the several periods in 4 tests on R. R. L. and in 5 tests on D. E. S. B. are given in table 4. And the original records for the preliminary period and for periods II and VI of the experiment on D. E. S. B. October 25, 1924, are reproduced in figure 5. Examination of tables 3 and 4 reveals the usual differences to be noted between the taking of cold water, or a combination of cold water and ice, which establishes a considerable heat debt, and the taking of an approximately equivalent amount of water at a temperature which makes no noteworthy demand for extra heat production. Except in one case which was defective, the lowest figure for the estimated calories per square meter of body surface in the preliminary tests was taken as basal. The average heat debt established in 22 cases was 449 small calories per kilo, with variations between 350 and 527. In these cases the average increase of metabolism during the period of greatest effect was 16.4 per cent, with variations between 8.4 and 38. There was no close relation between the amount of the heat debt and the increase of metabolism, though in four cases, in which the debt was below 400 (averaging 375 calories per kilo), the average increase of metabolism was only 11.4 per cent. On the other hand the two lowest increases, 8.4 and 8.5 per cent, occurred when the debt was more than 500 calories per kilo. In one of these cases, the room tem-

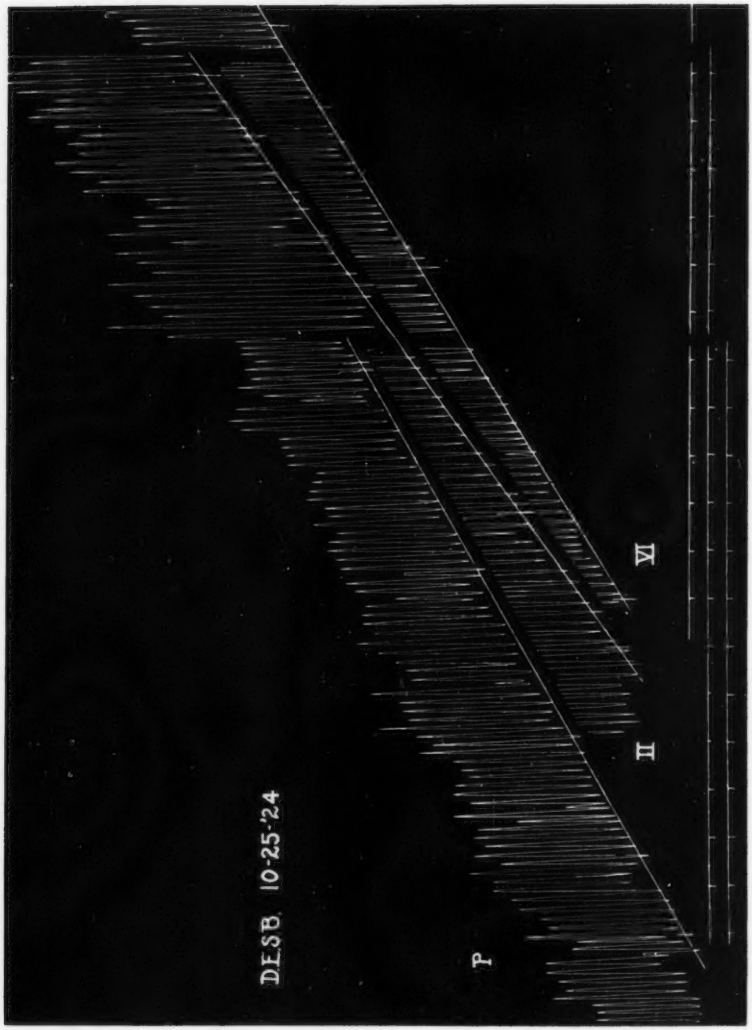

Fig. 5. Records of the preliminary period, *P*, and of periods II and VI of the observations on D. E. S. B., October 25, 1924 (see table 4). Windows have been cut in P and II to permit the base line of the underlying records to be seen. Time in half-minutes. In each case the record ran for at least six and a half minutes. Original record reduced one-third.

perature was unusually high (28°), a circumstance the importance of which has already been noted and which will be further emphasized later; and in the other case there may have been a depressed condition such as occurs occasionally and may occur regularly, in which cold is not reacted to (see p. 496). The greatest increases were seen when the debt was between 400 and 500; in sixteen such instances it averaged 18.6 per cent.

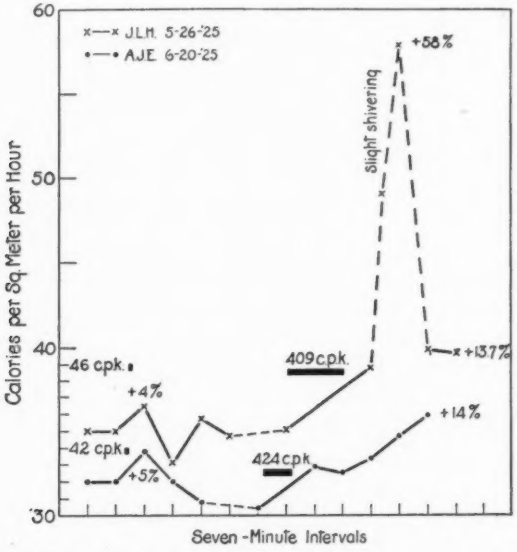

Fig. 6. Changes in metabolism produced by taking warm water and later by taking cold water and ice. A heat debt of 46 small calories (per k.) due to taking 520 cc. of water at 30° caused in J. L. H. an immediate increase of 4 per cent in the metabolic rate; a heat debt of 409 calories from taking 354 cc. of water at 1° and 130 g. of ice caused an increase of 13.7 per cent. Slight shivering was attended by a sudden and temporary increase of 58 per cent. A heat debt of 42 calories from 420 cc. of water at 30.2° caused in A. J. E. an immediate increase of 5 per cent in the metabolic rate; a heat debt of 424 calories, from 260 cc. of water at 1° and 139 g. of ice, caused a gradual increase of 14 per cent, without shivering. Each point breaking the line represents a metabolism record.

Although in all these instances there was, of course, some disturbance of the subject when the cold water was given, the time of highest metabolism was not immediately thereafter, but regularly much later—averaging 23 minutes, i.e., in the third or fourth period.

The results of control observations were markedly different. The water was given at a temperature near 30°. The average heat debt in

TABLE 5

Cases of depression of metabolism caused by a heat debt. The axillary temperatures are reported in parentheses

DATE	SUBJECT	SEX	AGE	WEIGHT	HEIGHT	TEMPERATURE OF ROOM	AMOUNT OF		HEAT DEBT	METABOLISM—CAL. PER SQ. M. PER HOUR								MAXIMUM DEPRESSION BELOW BASAL	MAXIMUM RISE ABOVE BASAL
							Ice	H_2O		Preliminary	I	II	III	IV	V	VI	VII		
				kgm.	*mm.*	*°C.*	*gms.*	*cc.*	*cal./kgm.*									*per cent*	*per cent*
10/18/24	E. M. B.	♀	32	64.0	172.0	19.5	147	452	521	31.3 (*35.9*)	32.0 (*35.3*)	27.4 (*34.9*)	30.8 (*34.8*)	29.7 (*34.8*)	31.1 (*34.9*)	30.7 (*34.9*)	31.9	−12.5	±0.0
10/30/24	E. M. B.					22.5	138	413	476	32.1 (*36.2*)	31.3 (*36.2*)	31.5 (*36.0*)	33.4 (*35.9*)	32.6 (*35.9*)	32.3 (*35.9*)			−2.5	+4.0
2/19/25	E. M. B.					20.0	147	420	489	30.2 (*36.0*)	32.6* (*36.1*)	29.9 (*36.0*)	30.4 (*35.9*)	31.9 (*36.0*)	33.2 (*36.0*)			−1.0	+10.0
3/17/25	E. M. B.					19.5	120	226	323	31.8 (*36.1*)	31.6 (*36.0*)	29.9 (*36.0*)	32.3 (*36.0*)	33.9 (*36.0*)	33.4 (*36.0*)	31.2 (*36.0*)	30.5 (*36.0*)	−6.0	+6.5
5/19/25†	E. M. B.					*22.8*		*603*	*150*	*29.6* (*36.2*)	*28.7* (*36.0*)	*28.0* (*36.0*)	*31.1* (*36.0*)	*29.7* (*36.0*)	*28.7* (*36.0*)			*−5.5*	*+5.0*
10/ 7/24	S. W. B.	♂	32	73.0	177.0	21.5	136	500	464	41.1 (*36.6*)	34.1 (*36.4*)	34.2 (*36.3*)	41.1 (*36.3*)	43.4 (*36.4*)	39.5 (*36 5*)			−17.0	+5.6
4/15/25	J. L. H.	♀	25	68.0	164.5	20.5	120	400	384	30.6 (*35.5*)	29.9 (*35.8*)	34.0 (*35.8*)	33.2 (*35.8*)	33.1 (*35.8*)				−2.4	+11.0
3/17/25	J. R. L.	♂	26	73.8	177.5	19.2	143	495	435	41.5 (*35.6*)	35.9 (*36.0*)	40.3 (*35.9*)	41.8 (*35.8*)	43.4 (*35.7*)	40.3 (*35.8*)	42.5 (*35.8*)	38.5 (*35.7*)	−13.5	+4.5

* Rise due to activity of taking ice and H_2O.

† *Control.*

TABLE 6

Cases showing the marked increase of metabolism which occurs when shivering appears. The presence of shivering is indicated by an asterisk (*). The axillary temperatures are reported in parentheses

DATE	SUBJECT	SEX	AGE	WEIGHT	HEIGHT	TEMPERATURE OF ROOM	AMOUNT OF		HEAT DEBT	METABOLISM—CAL. PER SQ. M. PER HOUR								TIME OF MAXIMUM METABOLISM AFTER H_2O	INCREASE IN METABOLISM
							Ice	H_2O		Preliminary	I	II	III	IV	V	VI	VII		
				kgm.	*mm.*	*°C.*	*gms.*	*cc.*	*cal./kgm.*									*min.*	*per cent*
1/29/25	E. M. B.	♀	32	64.0	172.0	16.6	145	545	565	31.4 (*36.2*)	36.4 (*35.9*)	41.3* (*35.9*)	37.4 (*35.9*)	34.0 (*35.9*)	32.1 (*35.8*)	30.5 (*35.8*)	31.3 (*35.7*)	18	31.5
1/22/25	S. W. B.	♂	32	73.0	177.0	14.4	143	520	476	41.1 (*36.7*)	53.0 (*36.25*)	59.1 (*36.25*)	79.7* (*36.35*)	67.8 (*36.5*)	67.1 (*36.6*)	52.8 (*36.7*)	40.3 (*36.75*)	29	94.0
3/14/25	A. J. E.	♀	29	60.0	168.7	20.0 18.0	161	266	464	32.9 (*36.0*)	46.9* (*35.7*)	38.0* (*35.5*)	37.2 (*35.5*)	36.1 (*35.5*)	36.6 (*35.5*)	35.6 (*35.5*)		7	42.2
3/23/25	A. J. E.					19.0	150	272	453	31.9 (*36.3*)	42.3* (*35.3*)	42.7* (*35.6*)	39.1* (*35.65*)	38.2 (*35.7*)	36.7 (*35.8*)			5	34.0
2/ 2/25	A. T.	♀	49	66.0	171.3	18.6 17.0	147	435	492	35.7 (*36.0*)	42.1 (*36.3*)	43.5* (*36.1*)	46.5* (*36.0*)	41.7 (*36.0*)	?	35.8 (*35.9*)		23	30.2
1/22/24	A. Q.	♂	23	54.2	156.2	18.5		665	454	37.8 (*37.2*)	40.4 (*37.2*)	60.0* (*36.9*)	52.1* (*36.8*)	44.7 (*36.8*)	45.8 (*36.8*)	41.2 (*36.8*)	42.9 (*36.8*)	14	59.0

11 cases was 50 small calories per kilo, a little more than a tenth of the debt when the cold tests were made. In one case no change of metabolism occurred, in another it fell 1.3 per cent; and the average of all the cases was an increase of only 3.1 per cent. Furthermore, this increase was probably largely caused by the disturbance due to taking the water, for in all but one case it came in the first period thereafter, instead of the third or fourth period, which was the time of highest metabolism after the cold intake. In three cases the control observations were followed in a short time by observations on the effects of cold water—A. J. E., June 20; J. L. H., May 26; and J. R. L., September 28, in table 3. The results in two cases are displayed graphically in figure 6. In these three instances the taking of warm water, comparable in amount with that taken as cold, resulted in only relatively slight increases of metabolism; whereas the taking of cold water and ice after a short period of rest, but otherwise under the same circumstances, resulted in typical accelerations of the metabolic rate.

The phenomenon of depression, or of failure to "react," after exposure to cold (e.g., after a cold bath) is well known. In one of the subjects (E. M. B.) depression, in the sense of feeling miserable and ill and also in the objective sense of a lowered metabolic rate, was a characteristic result of taking ice and ice water. The figures for the tests made on her are given in table 5. It will be noted that in the one test in which she showed clear acceleration of metabolism she shivered (see table 6). It is noteworthy also that she had an abnormally low metabolic rate—in six tests varying only slightly from an average of 31 calories per square meter per hour, which was 15 per cent below the standard. Compare also J. L. H. in tables 3 and 5. On the other hand, even in persons who usually reacted to the heat debt by prompt responses which were directed towards payment, the metabolic rate might be depressed in occasional experiments, as shown in the other subjects of table 5. In all these cases the depression was temporary, and was followed by a rise of metabolism, often to a level well above that which prevailed in the preliminary (basal) period.

Table 6 is presented to show that when shivering occurs the metabolic rate is, usually, much more strikingly accelerated than when, as in the cases shown in table 3, it is absent (see also J. L. H. in figure 6). Shivering causes a marked and sudden increase of metabolism, an increase which may be as much as 60 or 90 per cent above the basal rate. In the observation on E. M. B. January 29, 1925, the increase of 31.5 per cent does not appear great as compared with the increases in the other subjects. The results in that instance, however, should be compared with those from E. M. B. in table 5. It will be seen that her most characteristic reaction to the cold was depression. The increase of 31.5 per cent when

TABLE 7

Cases showing the influence of external temperature on the efficacy of a heat debt in causing increase of metabolism. The axillary temperatures are reported in parentheses

DATE	SUBJECT	TEMPERATURE OF ROOM	HEAT DEBT	METABOLISM—CAL. PER SQ. M. PER HOUR							TIME OF MAXIMUM METABOLISM AFTER H_2O	INCREASE IN METABOLISM
				Preliminary	I	II	III	IV	V	VI		
		°C.	cal./kgm.								min.	per cent
10/28/24	J. R. L.	25.0	450	37.0 (36.1)	37.0 (35.8)	37.7 (35.8)	37.2 (35.8)	37.6 (35.8)	38.2 (35.7)	38.8 (35.8)		
1/22/25	J. R. L.	14.0	470	39.0 (35.8)	46.9* (35.4)	44.3* (35.5)	46.3* (35.5)	48.8 (35.5)	50.9 (35.5)		41	30.0
10/30/24	S. W. B.	23.3	480	43.4 (36.4)	43.8 (36.1)	43.8 (36.1)	44.4 (36.0)	44.4 (36.0)	43.9 (36.1)	42.1 (36.1)	25	2.4
1/22/25	S. W. B.	14.4	476	41.1 (36.7)	53.0 (36.25)	59.1† (36.25	79.7† (36.35	67.8† (36.5)	67.1† (36.6)	52.8 (36.7	17	28.5‡
6/ 4/25	J. D. P.	27.7 28.5	527	39.4 (36.4)	40.3 (36.2)	40.4 (36.1)	41.9 (36.1)	38.6 (36.1)	39.4 (36.3)		24	6.2
4/14/25	J. D. P.	20.0 21.5	485	40.4 (36.4)	41.4 (36.0)	47.8 (36.0)	55.7 (36.0)	52.9 (36.0)	48.0 43.3 (36.1)	41.8 (36.2)	14	38.0

* "Waves of cold" felt in back. No shivering observed.

† Shivering.

‡ Calculated on 53.0.

she shivered was quite remarkable. The increase of 30 per cent shown by A. T. during shivering was more than twice that shown by her when she did not shiver (see table 3).

If the external air is colder than the body there is, of course, a flow of heat to it from the body—a flow which is controlled to a large extent by the degree of exposure of warm blood in surface vessels. There is evidence that when conditions tend to lower body temperature the surface vessels are contracted (Tigerstedt, 1923). Thus heat is conserved. When a heat debt is established by taking cold water and ice, the debt may be at least partially paid by retaining the heat which otherwise would be lost through the skin. If the external air is sufficiently cold, however, the agencies for heat conservation may already be working at a maximum. In that event the only manner of meeting the debt would be by extra heat production. The condition is quite otherwise if the external air is warm and the surface vessels are dilated in order to increase the heat loss. In these circumstances, extra heat production may not be necessary if the escaping heat is saved and applied to paying the debt. In cold surroundings, therefore, a given heat debt might be expected to cause a much greater increase of metabolism than it would cause in warm surroundings. That such is the case is shown in table 7. In the case of J. D. P., for example, a heat debt of 527 calories per kilo increased the metabolism only 6.2 per cent when the temperature of the air was about 28°, whereas a debt of 485 calories induced an increase of 38 per cent when the temperature was about 21°. In the cases of J. R. L. and S. W. B. in table 7, "waves of cold" or shivering were induced by the heat debt, and the metabolism was markedly increased even in the absence of these conditions when the external temperature was low; whereas nearly the same heat debt had no such effects when the external temperature was 9° to 11° higher. It is evident that there may be difficulty in obtaining such results as are reported in table 3 if the experiments are carried on in very warm surroundings.

The results of our studies on cats showed that a considerable heat debt, which fails to cause shivering, still causes an increased discharge of adrenin, that when shivering occurs the extra adrenin output precedes it, and that when adrenin is excluded from action the organism resorts to shivering as it does not when adrenin is present. These observations disclose the importance of adrenin as a calorigenic factor and indicate that for the chemical increase of heat production the organism relies upon the adrenal medulla primarily. Adrenin acts by raising the metabolic rate. In the foregoing experiments on human subjects (table 3) the metabolic rate was raised, without shivering, under conditions precisely resembling those which evoked in lower animals a greater adrenin discharge. We conclude, therefore, that in man as well as in lower animals

the adrenal medulla is stimulated by a threatened fall of body temperature and that its secretion, by hastening combustion, acts to avert the danger.

Discussion. Rubner (1902, p. 221) has defined chemical regulation of body temperature as the maintenance of the normal temperature by "increase of heat production in the resting animal." For many years there has been a controversy concerning a true chemical regulation in Rubner's sense. One group of investigators has contended that the increase of heat production in time of need is wholly due to the effects of shivering or of greater muscular tension, i.e., the animal is not "resting." The other group has maintained that an increased heat production may occur with no muscular activity whatever to account for it.

The view that chemical regulation of temperature is accomplished solely by the additional metabolism that results from shivering or other muscular contraction has been supported by Speck, by Loewy, by Johansson, and by Sjöström. Speck (1883) placed himself in water at 20–21.5°C. for 10 to 12 minutes and recorded his respiratory exchange; he inhibited all muscular action and then observed no certain influence of the external cold on his metabolism. Loewy (1890) made 55 observations on 16 persons who for 2.5 to 3 hours were exposed to cold. The oxygen consumption was constant in 20 cases, was less in 9, and was greater in 26 (47.3 per cent). The body temperature fell in nearly all the tests and there was a sensation of cold. Whenever the oxygen consumption was unchanged or decreased, there was complete quiet. Of the 26 instances of greater oxygen usage, in 13 there was obvious shivering, in the other 13 Loewy suggested, as possibly accounting for the results, unobserved shivering, minor muscular contractions, or continuous slight tensions without visible movements. It is noteworthy that in one instance an increase of 33.3 per cent occurred in the oxygen utilization without visible shivering. Loewy's conclusion, however, was that extra heat production, if called forth by cooling, is dependent on muscular activity. In the experiments of Johansson (1897) the CO_2 output was measured as subjects were exposed to cold air in a respiration chamber. The tests on himself lasted 2.25 hours—a half-hour as a preliminary period, a quarter hour for undressing, *one hour* for exposure, naked, to the cold (12.6° to 18° on different occasions), and a half-hour as an after-period, dressed. The average CO_2 output per hour was greater during the cold period than before or after, but not in accordance with the temperature differences. Shivering occurred during some of the tests and then the output might be increased to threefold that of the preliminary period. When all muscular movements were voluntarily suppressed, Johansson states, no sign of chemical heat regulation was observable. He concluded that chemical heat regulation without muscular contraction does not exist.

Sjöström (1913) using the method employed by Johansson, and studying, on clothed or naked subjects, only those responses which occurred reflexly or automatically during a two-hour exposure to cold air, found that the metabolism, as measured by the CO_2 elimination, is independent of external temperature, so long as shivering is absent. When shivering occurs it increases the CO_2 production somewhat, but the greater oxidation represented by it may not be sufficient to prevent a considerable drop in body temperature. Since shivering may appear suddenly after a brisk cold douche when a noteworthy fall of temperature cannot have taken place, or may appear when one removes one's clothing and still has a rectal temperature within the normal range of variation, Sjöström argues that shivering is a reflex initiated by the cold receptors, and that therefore the temperature conditions of the skin are decisive for its onset.

Opposed to the evidence that all chemical regulation of heat production involves muscular movement are the observations of Voit and of Rubner. Voit (1878) reported experiments on a clothed man in the condition of basal metabolism who was exposed for six hours to temperatures ranging between 4.4° and 30°; at the end of the experiment with the lowest temperature (4.4°) he shivered, but the CO_2 output at 6.5°, without shivering, averaged 8.5 grams per hour more than at 14.3°. Voit argued against explaining this effect by reflex excitatory influence on respiration, and attributed it to a stimulating action of the nervous system on muscular metabolism similar to that occurring in muscular work (Voit, 1881). Voit's results were confirmed by Rubner and Lewaschew (1897) in tests on subjects who rested quietly from *five* to *eight hours* in surroundings where the temperature and water vapor could be controlled. They found that 32.34 grams of CO_2 per hour were given off in dry air at 15°, as contrasted with 27.9 grams at 23°; and in moist air at the same temperatures 34 grams as contrasted with 28.61 grams. Rubner also reported similar observations on dogs. In one instance, for example, the heat production rose from 64.5 Calories per kilo for 24 hours at 31° to 83.1 Calories at 15.5° and to 120.5 Calories at 7.3°. He comments (1902, p. 138): "It is incomprehensible that many physiologists can still doubt the presence of this powerful regulating mechanism. And it is just as incomprehensible that the increase of heat in the presence of cold is ascribed solely to the gross shivering of the animal. Will anybody assume that animals which show chemical regulation at 30° begin to shiver at 29°, 28°, etc., when they withstand a temperature of 0° without any visible disturbance?" The most recent supporters of the view that there is a true chemical stimulant of metabolism when extra heat is needed are Leonard Hill and his collaborators (1921). They exposed subjects to surroundings which had different cooling powers, but did not permit the cooling to continue to the point at which shivering appeared. The ex-

pired air was collected and analyzed. The data thus obtained showed that the metabolism was increased directly with the cooling effects of the atmosphere.

Here is a sharp difference of evidence and of inference that demands explanation. What may be said regarding the contradictory results?

1. The experiments said to prove that shivering is essential to increased heat production are not wholly consistent, and therefore not decisive. This is a criticism which Rubner made with reference to both Loewy's and Johansson's studies. Of Loewy's 55 cases we need consider only those in which cold increased the metabolism, as shown by a greater oxygen usage. In 50 per cent of these cases there was no obvious shivering: Loewy had to *assume* that muscular contractions and tensions were present—he did not prove their existence. If they were not present the augmented metabolism must have been due to some other factor. Again, in Johansson's personal experiments, although a three-fold increase of the CO_2 output was associated with shivering in one instance (a degree of effect never observed by Rubner), considerable increases over the figures for the preliminary period—as much as 11 and 28 per cent—were reported, with testimony that there was "no shivering." Sjöström's observations, likewise, revealed a larger output of carbon dioxide during the second hour of exposure to cold—in one case a rise of 7 per cent, in another 14 per cent, without shivering to account for it. It is clear that such conditions as these, occurring in the work of investigators who have interpreted their results as demonstrating that chemical increase of heat production without muscular contraction does not exist, permit us to question that conclusion.

2. The presence of shivering does not exclude the additional presence of a pure chemical stimulus to extra heat production. Indeed, as shown in figure 1, chilling an animal with cold air may cause shivering, but simultaneously the chilling augments adrenal secretion, as shown by acceleration of the denervated heart. Indeed, all our evidence supports the conclusion that when conditions are such as to induce shivering, they are also such as to induce a faster rate of adrenal secretion. Extra heat production as a consequence of a larger output of adrenin would therefore coexist with the extra heat production due to muscular activity. This fact at once vitiates the conclusion that shivering alone accounts for the chemical regulation of body temperature.

3. Subjects exposed to cold for *short* periods, and subjects who, having become "hardened," do not react to cold as formerly, may not shiver when exposed to heat loss and may not manifest any other sign of faster metabolism. It is noteworthy that both Voit's and Rubner's experiments lasted many hours, while those of their opponents lasted a much shorter time. As Rubner has pointed out, in *short* experiments there may

not be sufficient heat loss to call the calorigenic apparatus into action. It seems to us that this condition is especially apt to occur in such experiments as Speck's in which the body is exposed to cool surroundings for only a few minutes. Further, the specific heats of air and the body are so different that *long* experiments in cold air might be required before the mechanisms for augmenting heat would be set in operation. Especially would this be true as the subjects became accustomed to the cold surroundings. Both Loewy and Johansson noted this "hardening" of their subjects, so that an external temperature that at first caused marked shivering later had no effect at all. The nature of this adaptation is not clear. It may be an adjustment of the skin receptors so that they become less sensitive to external cold; it may be a better and quicker action of the vasoconstrictor mechanism, which, checking the flow of warm blood in the surface vessels, protects the body against heat loss. Whatever its nature, however, it may be associated not only with absence of shivering, but also with absence of true chemical calorigenesis. Then the O_2 intake, as in Loewy's observations, or the CO_2 output, as in the observations of Johansson and Sjöström, may remain almost unchanged during exposure to cold for the relatively short periods of their experiments. Such evidence has the weakness of all negative evidence, and is of little value in the presence of positive evidence to the contrary. In short, the mere fact that the chemical mechanism for producing extra heat is not brought into operation is no proof that it does not exist.

Our criticisms of the opponents of true chemical heat regulation may be summarized as follows: in experiments on the effects of cold they have reported substantial increases of metabolism that had no shivering to account for them; they have failed to recognize that shivering may coexist with and effectively mask a true chemical calorigenesis; and they have inferred that because a chemical mechanism did not operate in certain short experiments the mechanism does not operate—an inference having slight value when confronted with good evidence to the contrary.

It is obvious that the objections raised against short experiments on the effects of exposing the body surface to cool air or to moderately cool water cannot be raised against our experiments as reported in the foregoing pages. In them a large amount of *cold* water was introduced into the stomach. It has a higher heat capacity per unit quantity than the body itself. It was at or near 0°. It would represent a considerable heat deficit if no reaction should occur. Great vessels and highly vascular organs (the liver and spleen) are near, so that the organism cannot prevent escape of heat into this water, as it can prevent escape of heat into the surrounding cool air, by vasoconstriction. It can save some of the heat it is losing externally and this may be applied internally to the task of warming the water. But in the main the body has only one protection

against the cold mass in the stomach. It must produce more heat. That this condition is associated with increased adrenal secretion—a secretion which has calorigenic powers, that with this mechanism in action shivering may not be relied upon, and that in the absence of shivering the metabolism is considerably heightened by cold, we have shown by our experiments. They definitely support the testimony and the arguments of Voit and Rubner, and offer an explanation of the chemical regulatory device which they postulated.

Evidence recently reported by Hunt and Bright (1926) has shown that adrenalin increases metabolism by action on both the viscera and skeletal muscles. The effect of curare in lowering or abolishing the ability of the organism to maintain its temperature has been used as proof that the chemical agencies for heat production reside in muscles and are subject to the direct nervous control which makes muscles contract. This effect of curare, however, should not be regarded as evidence against the calorigenic service of increased medulliadrenal secretion. Seven years ago, when it seemed possible that a rise of blood pressure from constriction of a denervated splanchnic area could be used to demonstrate reflex adrenal secretion, curare was given by one of us to eliminate disturbing muscular movements. It was soon apparent that the amount of curare that blocked nerve impulses to skeletal muscles greatly reduced the responses from the adrenal medulla. Stewart and Rogoff (1919) have noted effects leading to the same conclusion. Curare would tend, therefore, to stop acceleration of metabolism whether due to increased output of adrenin or to muscular contraction.

In connection with the two modes of meeting a heat deficit—by augmented discharge from the adrenal medulla and by shivering—it is interesting to note the manner in which the organism meets the critical situation presented by hypoglycemia. Cannon, McIver and Bliss (1924) have called attention to two lines of defense which arise when dangerous hypoglycemia is threatening; first, an excitation of the sympathetic system, with attendant larger medulliadrenal secretion, which calls forth sugar from the liver, and second, a convulsive attack, or series of such attacks, in which the sympathetic discharges reach an acme. From the facts presented above it would appear that likewise when there is danger of a lower body temperature from heat loss, a first defense is set up by bringing into action the sympathetic system. Hairs or feathers are lifted till a thicker layer of poorly conducting air is enclosed about the body, peripheral blood vessels are constricted so that the escape of heat by radiation and conduction is diminished, easily combustible sugar is liberated into the blood stream, and adrenin which accelerates combustion is put forth in greater amount. Again, if this first line of defense fails, and the temperature tends to drop in spite of it, there is a second line in

the control of muscular action by the nervous system—shivering is evoked. And just as the convulsive seizures in hypoglycemia are accompanied by a greater discharge of adrenin, so likewise is shivering due to cold (see p. 473). In both circumstances introduction of the muscular factors marks a climax of the action of the automatic arrangements for defense.

It seems probable that the chemical mechanisms for heat production rarely come into service in the ordinary comfortable existence of civilized man. Persons who live through a winter in houses and public buildings, often kept unpleasantly warm, are subjected to few of the exigencies which their ancestors had to meet under primitive conditions. Even if a superficial feeling of being cold signals a possible danger of heat loss, warm clothing is at hand to protect against an amount of heat loss that would call the mechanisms into action. And furthermore, as a rule, the heat produced as a by-product of ordinary functioning of muscles and glands, when associated with quick voluntary protection against heat loss, would be adequate for the ordinary demands of cold weather as most of us have to meet them. If the artificial protection is lacking, however, or if it proves unequal to the occasion, the heat-producing mechanisms are ready, and can be brought into service promptly to help in the emergency.

SUMMARY

To indicate increased secretion of adrenin the completely denervated heart was employed. Since the thyroid gland and the liver were likewise denervated, the adrenal medulla alone was left as a factor capable of causing, humorally, a faster heart beat. The animals thus prepared lived normally in the laboratory.

The first test applied was exposure of the animals to a cold environment. Under these conditions, with no emotional element or struggle involved, the rate of the denervated heart increased between 18 and 33 per cent. After the right adrenal gland was removed and the left was wholly denervated, the same test on the same animals caused the heart rate to decrease (see fig. 1).

The second test was the establishment of a heat debt by introducing a known amount of cold water (between 0° and 1°) into the stomach—a method which is easily available at any time and, with fairly uniform environmental temperature, is satisfactorily quantitative (see p. 471). The heat debt established in cats varied between approximately 1500 and 2000 small calories per kilo (see table 1). There was an initial disturbance of the heart rate due to giving the water, but after that subsided to a plateau, the elevation of rate ranged between 23 and 28 per cent. This increased heart rate may continue for an hour or more (see table 1, fig. 3 *B* and fig. 4). When equal amounts of warm water (between 31° and 34°) were given, the increased rate disappeared in fifteen minutes

or less (see table 1 and fig. 3 *C*). After the adrenal glands had been rendered inactive the test with cold water on the same animals caused only slight acceleration and in some instances depression of the heart rate (see table 1, fig. 3 *D* and fig. 4).

From these observations the conclusion is justified that conditions which would naturally cause a lowering of body temperature induce an increased discharge of adrenin into the circulating blood.

The evidence that secreted adrenin has a calorigenic action is reviewed and two types of experiment are proposed to demonstrate its service to the organism—*a*, its relation to shivering, and *b*, its effect on metabolism in the absence of shivering.

When the heat debt is large (1000 small calories per kilo or more, with the water at 1°) and the room temperature is about 20° it is commonly met by two calorigenic agencies—increased output of adrenin and shivering. Although shivering coincides with the period of greatest medulliadrenal discharge (see fig. 1), it is not a necessary condition for the discharge—it may be wholly absent though the heart rate is well accelerated (e.g., no. 26, March 16, table 1).

If in an environmental temperature of about 20° a heat debt of only 900 calories is to be paid, shivering rarely occurs; and if it occurs, it is of short duration (3 minutes). If now the adrenal glands are put out of function, and a heat debt of 900 calories is established, shivering almost uniformly occurs and may last for as long as 17 minutes (see table 2). Thus when the heat-producing service of the adrenal medulla is lacking, the shivering mechanism is resorted to.

Establishing a heat debt averaging 449 small calories per kilo caused in twenty-two observations on eleven human subjects, an average maximal increase of metabolism of 16.4 per cent, with variations ranging upward as high as 38 per cent. These increases were not accompanied by shivering. The development of the peak of the metabolic rate averaged twenty-three minutes after the cold water was taken, and therefore was not due to the disturbance of taking the water. When an equivalent amount of warm water was taken the average increase of metabolism was only 3.1 per cent, and since the time of maximal increase occurred regularly in the first seven minutes of the experiment, it must have resulted chiefly from the disturbance of taking the water (see tables 3 and 4 and also figs. 5 and 6).

In one of our cases regularly and in others occasionally the heat debt induced a preliminary fall of metabolism which was followed by a rise often to a level well above that of the preliminary basal period (see table 5).

When shivering occurs it usually causes a marked and sudden increase of the metabolic rate, which may be as much as 60 or 90 per cent above the basal (see table 6 and fig. 6).

In cold surroundings a given heat debt causes a much greater increase of metabolism than in warm surroundings (see table 7). Our experiments have shown that the same conditions which increase adrenal secretion in lower animals increase metabolism in man, and this without shivering. We conclude, therefore, that a disturbing heat loss evokes activity of the adrenal medulla and that the extra output of adrenin by hastening combustion, serves to protect the organism against cooling.

The experiments of Voit and Rubner and of their opponents are discussed in the light of our experiments. Our experiments obviously have an important bearing on the long-waged controversy over the question of true chemical augmentation of metabolism. They support the contentions of Voit and Rubner that such a process exists in the body and they account for the mode of action of that process.

The effects of curare are considered, and also the rôle of medulliadrenal secretion and of shivering as primary and as secondary defences of the organism against a fall of body temperature.

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STUDIES ON ADRENAL INSUFFICIENCY

III. The Influence of Pregnancy upon the Survival Period in Adrenalectomized Dogs[1]

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Statements as to the influence of pregnancy upon the period of survival after adrenalectomy, based upon observations on such animals as rats or rabbits are of no value, since it cannot be known whether, in the absence of pregnancy, the animal might not have lived just as long. On the other hand, dogs are extremely suitable for such observations since they all die, and in a relatively short time, after removal of both adrenals. In paper I of this series (Rogoff and Stewart, 1926a) it is shown that of 74 male and non-pregnant female dogs two lived into the 15th day; a number lived 10 or 12 days; the great majority 4 or 5 to 8 or 9 days, and a few less than 4 days. By comparing the survival periods of a number of pregnant dogs with these results it is easy to see whether the pregnant condition is associated with any marked departure from the normal. Table 1 shows what happened in 17 pregnant dogs in which the adrenals were removed at 2 operations. In some of the animals impregnation occurred before removal of the first adrenal (e.g., dogs 1034, 1128, 1137, 1168, 1185, 1192 and 1198); in others, in the interval between the two adrenalectomies. In 3 of the dogs the time of coition was known, precisely in dogs 1162 and 1174; and to a day or two in dog 1036. In the last two cases the coition occurred about a week before the second operation; in 1162 more than 2 weeks.

The striking fact brought out in the table is the number of animals which lived far beyond not only the average of the control dogs (tables 1 and 2, paper I) but the extreme limits of their survival periods. Thus, dog 1179 lived till near the end of the 13th day; dogs 1176 and 1185 into the 15th day; dog 1026 into the 18th day; dog 1192 into the 23rd day; dog 1143 into the 26th day; dog 1034 almost 27 days; dog 1002 into the 33rd day; dog 1036 into the 47th day; dog 1157 into the 58th day; and dog

[1] An account of this investigation was communicated to the Federation of American Societies for Experimental Biology, December, 1925.

1174 into the 59th day. Putting the matter a little differently, out of 17 dogs, 8 lived more than 17 days and 5 about 13 to 15 days; whereas among the 74 controls (tables 1 and 2, paper I) not a single dog reached 15 days, and only 2 lived into the 15th day. The decisive difference, however, is the relatively large number of very long survivals (several or many weeks), a feature not seen at all among the controls.

It is true that dogs 1002 and 1026 were injected with Ringer-dextrose solution before it was shown that pregnancy influenced the survival period, and it is impossible to know whether the prolongation of life should be attributed to the pregnancy or to the injections. Even if these two animals are omitted, however, the demonstration remains complete that pregnancy in some way can make good the loss of the adrenals for a period sometimes approximately as long as the gestation period (about 60 days in dogs). Not only was life prolonged, but the animals remained in good health to within a day or two of death. In several instances it is probable that accidents connected with the pregnant state or with parturition helped to cut short the life of the animal. In one case (dog 1036) death was directly due to perforation of a large duodenal ulcer into the peritoneal cavity. In scanning table 1 the reader may observe that there are a number of survival periods which, far from being prolonged, are shorter than those of the majority of the control males or non-pregnant females. Thus, dog 1137 only survived the second operation 31 hours, dying after parturition. Here there is every reason to believe that whether the parturition was premature and induced by the operation, or not, it contributed to the early death of the animal. It must be recognized that while the pregnant state protects as regards adrenal insufficiency, it does not protect against the dangers of the operation as such, but itself constitutes an additional danger. Dog 1168, after recovering perfectly the day of the operation and remaining well on the next day, began to vomit blood-containing liquid on the second day after the operation. It is not known what the animal died of, but probably not, or not altogether, of adrenal insufficiency. It has seemed to us the only proper course to include all the dogs in table 1, leaving the reader in possession of the available data. With this object extracts from the protocols of some of the animals which outlived the second operation for the shorter periods will first be given. In some of these, blood studies were begun, including determination of non-protein nitrogen, etc., but the results are not reproduced in table 2, because the rapid development of the fatal symptoms did not permit a sufficient number of observations to be made.

Dog 1137. On 10/19/25 the left adrenal was removed. Body weight 10.85 kgm. 11/19/25, right adrenal excised at 11:15 a.m. Body weight 11.7 kgm. Excellent post-operative recovery, but at noon she vomited chocolate-colored material, and this was repeated at intervals. It contained blood.

11/20, in the morning she was lying on the floor; semi-comatose; raised her head on being spoken to but soon dropped it. Within an hour she had three yelling spells (labor pains?)and looked alarmed, attempting to run about in the cage. At 1:30 p.m. unchanged; yelling fits from time to time; involuntary defecation. At 2:30 p.m. in coma; tetanic spasms; at 6:00 p.m. coma and convulsions at intervals. At 6:45 p.m. she was found dead (warm, and rigor just beginning). One pup had been expelled and was lying near the vagina, another was bulging from the vagina. *Autopsy* at once. Plenty of fat. Ovaries large, with prominent corpora lutea. The uterus contained 6 pups, making 8 in all; not mature but near term. Milk could not be squeezed from breasts. Lungs and liver normal. Thyroids hyperplastic, moderately enlarged; thymus small. Pancreas markedly congested. Stomach, moderate congestion of mucosa near pylorus; two small ulcers at pyloric end. Small intestine, no congestion except in a few small spots; a little coffee-ground material (containing blood pigment) in lower end of ileum. Rectum moderately congested. Spleen normal. Kidneys moderately congested. Wound in good condition.

This is a practically negative picture, affording no explanation of the one unusual symptom, the vomiting of blood-containing material. The animal died in parturition, perhaps induced prematurely by the second adrenal operation.

Dog 1168. On 1/4/26 right adrenal removed. Body weight 10.4 kgm. 1/14, left adrenal excised at 2:30 p.m. Body weight 11.0 kgm. Post-operative recovery very good. On 1/15 the dog was in excellent condition, eating well. 1/16, in the morning was vomiting chocolate-colored material; this was repeated later; reaction of the vomit strongly acid to litmus; much blood pigment shown to be present. Food (meat) was refused.

1/17, at 2 a.m. condition unchanged. At 9 a.m. was found dead (still warm; had not been dead long). *Autopsy.* Lungs and heart, nothing special. Plenty of fat everywhere. Spleen normal. Liver and kidneys moderately congested. Thyroids not enlarged. Stomach, no decided congestion; contains 20 cc. of chocolate-colored liquid with one or two round worms. Much blood pigment. Small intestine, contains many worms but mucosa is not congested, and contents not bloody. Same for large intestine; one or two round worms even in colon. Pancreas decidedly congested. Uterus well advanced in pregnancy, though not nearly at full term; six pups in uterus. Mammae vascular, but milk could not be squeezed from nipples.

Dog 1198. On 4/22/26 blood was taken for analysis. On 4/26, the right adrenal was excised. Body weight 11.2 kgm. On 5/6 blood was taken for analysis. On 5/7 the left adrenal was removed at 10:30 a.m. Body weight 11.4 kgm. The post-operative recovery was excellent. On 5/8 seemed alright in the morning but refused bread and milk at noon. Later she took milk and some bread; but at 5 p.m. vomited bile, and seemed apathetic. 5/9. Livelier but not as brisk as before operation. Took some meat and milk. At 9 a.m. a blood sample was obtained for analysis. 5/10, unchanged. Ate fair noon meal (meat).

5/11. At 10 a.m. in getting a blood sample, it was seen that the flow was very slow and the blood dark. At noon food was refused. At 8 p.m. quite apathetic. On 5/12 she was apathetic but not asthenic. Took no food all day, but drank a good deal of water. Vomited bile. At 5 p.m. blood was obtained for analysis.

5/13, very apathetic; lying on floor, but when placed on legs can walk very well without wobble. At 5 p.m. a blood sample was got. At 8 p.m. unchanged.

On 5/14 at 6:10 a.m., found dead (still warm). *Autopsy.* Lungs, heart and kidneys

normal; spleen and liver somewhat congested. Pancreas very marked congestion. Stomach, very hemorrhagic, contains blood and bile. Mucosa of whole small intestine extremely hemorrhagic and contents almost pure blood. Same for large intestine. Uterus contained 5 pups (advanced pregnancy).

Dog 1128. On 9/29/25 the left adrenal was excised. Body weight 13.7 kgm.

11/3, seems to be pregnant. Right adrenal removed at 10:45 a.m. Body weight 15.1 kgm. Post-operative recovery very good; she was walking around at 11:00 a.m.

11/8. Condition excellent up to the present; she has eaten well; behaves normally; and is getting bigger. About 9 p.m. she had a coughing spell and between this and 2:00 a.m. (11/9) she coughed frequently and vomited. 11/9. Coughed and vomited (mucus) in the morning and again in the afternoon, but ate her noon meal (meat), and seemed in good condition. She is very heavy and the pups can be seen moving. Not at all asthenic.

11/10 (morning). She is very asthenic; can hardly stand and when attempting to walk wobbles in hind legs and falls over. Refused food (bread and milk) and water. At 4:50 p.m. panting respirations with occasional straining to expel the pups. At 4:55 p.m. a living pup was born; the sac was opened with scissors; the mother made no attempt to care for the pup. At 9 p.m. 2 more pups born. The mother is now better.

11/11. She took some water and milk; seems better and able to walk a little. At 11:30 a.m. began yelling and when the door was opened she rushed down the hall, yelling as if alarmed. In about 5 minutes she quieted down, and was apparently happy. At 1:00 p.m. she repeated the performance, racing about and barking violently for 5 minutes, and then quieting down and becoming friendly. Another barking fit and then she ate some meat. No asthenia. At 7:30 refused milk. Standing up. At 10:35 p.m. had 2 more living pups. She had a severe convulsion.

11/12. At 12:40 a.m. 2 more pups. She took milk. At 7 a.m. took a little milk and water. At 9:20 a.m. extensor convulsion, followed by coma. At 11:30 a.m. violent tetanic convulsion, followed by coma. At 1:00 p.m., lapped a little water. Died at 9:45 p.m. *Autopsy.* Plenty of fat. Thorax negative. Milk can be got from nipples. Pancreas decidedly congested. Stomach well filled with semi-digested food, which must have been there for 24 hours or more; no bile or blood; no congestion of mucosa. Some bile-colored liquid in duodenum and jejunum, but no blood and no congestion of mucosa. Lower third of small intestine contains some blood, and in parts the mucosa is congested. Large intestine no congestion, and no blood in feces. Omentum much congested. Liver congested.

As already stated, we cannot be certain whether any factor other than adrenal insufficiency was concerned in the death of the 4 animals hitherto considered. It is likely that in 1137 premature parturition caused by the second operation played an important part; and in 1168 the condition, whatever it was, with which the vomiting of blood was associated. Dog 1128 died in parturition at or about full term, more than 9 days after the second adrenalectomy. Here it is improbable that the second operation, as such, had anything to do with bringing on parturition, but it may very well be that the strain of parturition more than neutralized any protective influence of the pregnant state. In dog 1198, although the animal was in advanced pregnancy at the time of the second operation parturition did not occur. It is perhaps worth while to point out that the *post-mortem*

TABLE 1

Adrenalectomized pregnant dogs

NUMBER OF DOG	BREED	WEIGHT AT		TIME BETWEEN OPERATIONS	SURVIVED		BEGAN TO REFUSE FOOD	ADRENAL WEIGHTS		ALIMENTARY CANAL		PANCREAS CONGESTION
		First operation	Second operation					Right	Left	Blood	Congestion	
		kgm.	*kgm.*	*days*	*days*	*hours*	*days*	*grams*	*grams*			
996	Spaniel	8.0	8.7	19	15	2	$12\frac{1}{4}$	0.63	0.78	+++	+++	+++
1002	Airedale	11.6	13.3	13	32	$16\frac{1}{2}$	29	0.78	0.90	++	++	++
1026	Bull	11.5	13.3	39	17	6	$14\frac{1}{2}$	0.75	0.85	+++	+++	+++
1034	Collie	8.3	8.9	8	26	$18\frac{1}{2}$	26			+	0	0
1036	Bull	9.9	9.5	9	46	$3\frac{1}{2}$	44	0.76	0.67	+	+++	++++
1128	Collie	13.7	15.1	35	9	11	$8\frac{1}{2}$	0.90	0.72	+	+	+++
1137	Terrier	10.9	11.7	31	1	7	1	0.75	0.70	+	+	+++
1143	Collie	10.4	11.2	23	25	5	24	0.85	0.92	+	+	+++
1157	Collie	8.7	11.9	177	$57\frac{1}{2}$		57	0.57	0.78	+	+	0
1162	Bull	9.4	11.6	127	13	$7\frac{1}{2}$	$12\frac{1}{2}$	0.75	0.90	++++	++++	++++
1168	Shepherd	10.4	11.0	10	2	18	2	0.55	0.67	+	0	+++
1174	Fox terrier	6.2	8.7	120	58	3	55	0.40	0.50	+	0	++
1176	Collie	11.6	12.6	79	$14\frac{3}{5}$		$10\frac{1}{2}$	0.75		+++	+++	++++
1179	Shepherd	15.7	17.3	50	$12\frac{3}{4}$		$11\frac{1}{2}$	1.02	0.80	+++	+++	+++
1185	Spaniel	10.3	11.4	39	14	$3\frac{1}{2}$	12	0.64	0.70	++	++	++++
1192	Spaniel	9.7	10.4	19	$22\frac{3}{8}$		$19\frac{1}{2}$	0.80		+	++	+++
1198	Coach	11.2	11.4	11	$6\frac{1}{4}$		$3\frac{1}{2}$		0.85	++++	++++	++++

The right adrenal was excised first in all the dogs except 1034, 1036, 1128 and 1137.

TABLE 2

Blood analyses

NUMBER OF DOG	BLOOD TAKEN, DATE	PREVIOUS MEAL	NON-PROTEIN NITROGEN	UREA NITROGEN	URIC ACID	CREATININE		AMINO-ACID NITROGEN	UNDETERMINED FRACTION	DEXTROSE	Cl AS NaCl
						Preformed	Total				
1157	6–7	Bread, milk	27	9.0	2.5	1.1	2.1	10	6.5	0.10	510
	6–12	Bread, milk	32.6	10.5	1.5	1.1	3.0	9.3	11.3	0.11	495
	6–14	Bread, milk	28	10.5	1.3	1.1	2.0	10	6.4	0.08	495
	6–16	Bread, broth	27.3	10.7	1.4	1.1	3.1	8.2	7.6	0.09	510
	6–17	Meat	38	19	1.3	1.0	2 1	9.0	8.9	0.09	503
	6–18	Bread, milk	26	12	1.4	0.9	3.0	8.0	4.5	0.09	536
	6–19	Meat	33	14	1.4	1.1	2.4	9.2	8.5	0.09	510
	6–20	Bread, milk	27	13	1.3	0.9	2.2	9.0	3.8	0.11	495
	6–28	Meat	30	14.3	1.2	0.8	2.0	8.8	5.8	0.10	520
	6–30	Rabbit	31.5	15.8	1.1	1.1	2.0	9.6	5.1	0.10	503
	7–7	Meat, bread	28.6	13.7						0.11	510
	7–9	Meat, rabbit	27.3	14.3						0.18	520
	7–10	Milk, ham	33.3	19.3						0.13	510
	7–13	Meat	35.3	18.3						0.10	495
	7–15	Meat	33.2	16.2						0.12	528
	7–17	Meat, etc.	33.5	18.8						0.18	553
	7–19	Meat	37.5	24.2						0.11	528
	7–24	Meat	35.5	18						0.11	553
	7–26	Meat	36.3	20.8						0.10	503
	7–28	Bread, broth	38.7	21.4						0.08	544
	*7–28	Bread, broth	59	35.3						0.11	511
	7–29	Milk	75	54.5						0.12	528
	7–30	Meat	47.4	30						0.10	503
	7–31	Meat	85.7	54.5						0.11	503
	*7–31	Meat	118.8	66.6						0.11	478
	8–1	Meat	101.6	60						0.12	478
	8–2	Meat, milk	88.6	56.8						0.08	503
	*8–2	Meat, milk	78.3	46.5						0.09	487
	8–3	Meat	83.7	56.2						0.07	478
	8–4	Meat	60	38.4						0.08	495
	8–5	Meat	88.8	57.6						0.07	495
	8–6	Milk	148.5	77.4		1.3	3.5			0.07	528
	*8–6	Not eating	246	106			6.3			0.09	528
1162	4–27	Meat	41	19	1.0	1.2	3.7	14.0	6.4		454
	4–30	Bread, milk	30	10.7	1.4	1.2	3.0	8.8	9.0		503
	5–2	Meat	43	21.4	1.4	1.0	3.5	14.0	6.0		470
	5–4	Meat	32	15	1.3	0.8	2.7	10.3	5.4		478
	5–6	Meat	33	17	1.4	1.1	2.4	10.0	4.7		495
	5–9	Bread, milk	28	11.2	1.4	1.1	3.0	10.2	5.1		462
	5–11	Meat	44	22	1.3	1.0	2.8	9.3	11.3		462

* Blood samples collected in the evening; all others taken in the morning.

TABLE 2—*Concluded*

NUMBER OF DOG	BLOOD TAKEN, DATE	PREVIOUS MEAL	NON-PROTEIN NITROGEN	UREA NITROGEN	URIC ACID	CREATININE		AMINO-ACID NITROGEN	UNDETERMINED FRACTION	DEXTROSE	Cl AS NaCl
						Preformed	Total				
1174	5–6	Meat	43	26	1.3	1.0	2.3	9.3	6.5		528
	5–9	Bread, milk	27	11.1	1.4	1.0	2.8	10.2	4.3		495
	5–11	Meat	36	21.5	1.2	0.9	2.3	8.9	4.4		520
	5–14	Bread, milk	28.4	14.7	1.3	0.9	2.5	10.0	2.4		520
	5-20	Meat	46	25	1.2	1.0	2.4	9.4	10.4	0.08	520
	6–8	Meat	42.8	22		1.0	3.3	9.6	9.7	0.08	503
	6–14	Bread, milk	38	23.4	1.6	1.3	3.0	8.8	4.3	0.08	510
	6–28	Meat	48	37.5	1.4	1.0	2.4	8.8	0.4	0.07	553
	6–30	Rabbit	72.3	48.4	1.1	1.1	2.5	9.5	13.2	0.06	570
	7-1	Rabbit	42.8	24	1.2	1.0	2.1	8.9	8.8	0.08	560
	7–2	Rabbit	52.2	38.4	1.2		2.2			0.09	570
	7–3	Not eating	82	46.3	1.3	1.1	2.1	9.6	25	0.06	585
	7–4	Not eating	156	104	1.2	2.0	5.2	8.2	41.7	0.08	570
1198	4–22	Meat	38	19	1.5	1.1	3.0	13.3	4 2		510
	5–6	Meat	39	22	1.7	1.1	2.3	10.3	5.4		530
	5–9	Milk, bread	68	47	1.7	1.9	5.0	10.0	8.8		478
	5–11	Meat	60	37	1.7	1 2	2.7	10.0	11.5		410
	5–12	Not eating	86	52	2.0	1.5	3.7	11.6	20.0		430
	5–13	Not eating	146	92	3.1	2.3	6.5	13.0	37.5		430

changes in the gastro-intestinal tract which are so frequently found in the controls (paper I) were exceedingly pronounced in dog 1198 and quite trifling in the other three. While it would not be justifiable in the present state of our knowledge to say that the absence of these pathological changes makes it doubtful whether death has been caused solely by adrenal insufficiency, a glance at tables 1 and 2, paper I, will show how close the association is. In table 1 of the present paper there appears to be a relatively larger number of cases in which the gastro-intestinal changes are absent or slight. If that is not due to the table comprising too small a number of cases, it may indicate that pregnancy not only delays the appearance of the serious symptoms and staves off death after removal of the adrenals, but also in a certain number of cases prevents, or mitigates, the pathological changes in the mucosa of the stomach and intestines.

As for the group of 5 dogs (1179, 1162, 1185, 1176 and 996) which survived from $12\frac{3}{4}$ to over 15 days it might be difficult to conclude from them alone that pregnancy had a protecting influence. For scattered instances of about the same survival periods occur among the controls (tables 1 and 2, paper I). The distinguishing feature is that in the preg-

nant dogs there are 5 out of 17 with survival periods of this order of magnitude, a much higher proportion than among the controls. Some pertinent details are given in the following extracts from the protocols.

Dog 1179. 1/12/26, right adrenal removed. Body weight 15.7 kgm. Weight on 2/19, 16.7 kgm.; 2/24, 16.8 kgm. On 3/3, left adrenal excised at 10:35 a.m. Body weight 17.3 kgm. Animal obviously in advanced pregnancy. Post-operative recovery excellent. 3/4 and 3/5 condition very good, eating well. At 10:30 p.m. on 3/5 she was found cleaning up a litter of 8 (full term) pups, born between 7 and 10 p.m. Up to and including 3/14 she remained in good condition nursing and caring for four of the pups, the other four having died, possibly from being overlain. The mother ate well, was strong on her feet and walked normally. 3/15. Not eating; weak on legs (8 p.m.). At 12 m. was unchanged; lying with the pups.

3/16. At 6 a.m. dead (still warm). *Autopsy.* Pancreas congested. Stomach contains blood and bile; mucosa congested, hemorrhagic and bile-stained. Duodenum like stomach. Jejunum and ileum hemorrhagic, the congestion increasing in downward direction, but disappearing toward the lower end of ileum. Large intestine increasingly hemorrhagic from above downwards and contained blood. Cecum not congested, contained feces. Spleen and kidneys not congested. Lungs normal.

It is worthy of remark that this was a case in which the operation was performed almost at term, although there is no evidence that parturition was induced by the operation. The mother survived delivery between 10 and 11 days.

Dog 1185 was another case in which the second operation was performed in late pregnancy. Parturition did not begin till the 13th day after the second operation, and the mother never rallied completely. She was comatose at times, and was unable to give the usual care to the pups, although she had milk, and suckled them to some extent. In this dog it may be concluded that parturition was a contributory, perhaps the chief, cause of death. Had the exhaustion due to parturition been avoided, as possibly by a Caesarean operation, the protection against the consequences of adrenal insufficiency afforded by the pregnant state might have had an opportunity to show itself, as in dog 1143. In any case, had pregnancy been in an early stage, it is probable, as seen in so many of the early pregnancies, that the survival period would have been much lengthened, at least up to parturition. This may be the only reason why the protective influence seems to be more marked in animals which suffer the loss of the second adrenal soon after fertilization. But there may be another reason, which will be discussed in the proper place. It may also be pointed out, and illustrated by an example (dog 1176) that every case of early pregnancy does not necessarily survive for an extraordinarily long period after removal of the second adrenal. This animal indeed lived for $14\frac{2}{3}$ days, which is the extreme limit seen among the controls; but that is nothing compared with the survival periods of many of the

pregnant dogs. She was mated 11, 9 and 5 days before the second operation.

Dog 1185. 2/2/26, right adrenal removed. Body weight 10.3 kgm. Weights on 2/19, 2/24, 3/2, 3/4 and 3/10, 10.3, 10.8, 11.3, 11.4 and 11.9 kgm. respectively. On 3/4 left adrenal removed at 11 a.m. Post-operative recovery very good. For 12 days after the second operation she remained in excellent health, eating well and as active as dogs in late pregnancy usually are.

3/16. At 1:10 p.m. the dog was found lying on her side, semi-comatose and whining from time to time. Early in the morning she was in good condition and walked well. Even at 10:30 a.m., she was apparently well. Drew blood for analysis. At 3 p.m. she was yelling and the legs and head fixed in extension. At 4 p.m. deeper coma; pulse 92; respiration 40. At 7:30 p.m. she was conscious and wagged tail. At 9:30 p.m. a pup was born and at 9:45 p.m. another. The mother was taking a little interest in the pups, but not giving the usual care. Drank water.

3/17. At 9 a.m. she was nursing 5 pups. In addition 2 were dead.

On 3/18, at 12:30 a.m. she was in poor condition, dyspnoeic, semi-conscious, paying no attention to pups. Sometimes yelling loudly. At 10 a.m., comatose, sometimes in convulsions, which can be elicited by touching or tapping. Has passed liquid brown feces containing bile and much blood pigment. She died at 1:20 p.m. *Autopsy* at once. Plenty of fat. Thyroid normal. Thymus small. Lungs, liver and spleen normal, not congested. Uterus sub-involuted; breasts full of milk. Pancreas intensely congested. Stomach, mucosa hemorrhagic with many ulcers; contents clear, viscid liquid, blood stained. Duodenum, only slight congestion of mucosa in upper part and an ulcer extending through all the coats except the serous coat, opposite and above the main pancreatic duct. Jejunum and ileum free from hemorrhages and only a few small patches of congestion in the mucosa. Large intestine contains bloody feces; mucosa congested in places, increasing down towards the sigmoid. Cecum, no contents, but the mucosa is congested and hemorrhagic. Kidneys moderately congested. Enormous lymph glands on sides of iliac arteries.

Dog 1176. On 1/11/26 the right adrenal was excised. Body weight 11.6 kgm. On 3/10, 3/20, 3/26, 3/31 and 4/3 the weights were 11.8, 12.3, 12.4, 12,6 and 12.4 kgm. respectively. On 3/20, 3/22 and 3/26 she was successfully mated with a stock male dog.

3/31. Left adrenal removed at 1:30 p.m. Post-operative recovery very good. On 4/1 she was in excellent condition. On 4/2, eating voraciously; not distinguishable in behavior from normal dogs. 4/3 to 4/5. Unchanged . On 4/6 she vomited up her food but ate it again, later ate a good meal. On 4/7 not eating, but otherwise apparently in good condition. 4/8. Less active. Took 2 small pieces of meat, but refused more. On 4/9 ate a little meat; later vomited it. On 4/10 ate a small amount of bread and broth, but refused other food. At 9 p.m. seemed in very good condition; came out of cage and ate biscuit readily. On 4/11, taking no food; lies on bed most of time but is not weak when permitted to come out of cage. At 8 p.m. passed feces with bright red blood. 4/13, at 3:45 p.m. lying on bed; wagged tail. Has passed feces with brownish blood. Not in good condition. Same at 10 p.m. Took no food. On 4/14, took no food whatever, was very apathetic all day; lying on side; passed bloody feces; vomited bile in forenoon. At 10 p.m. unchanged. On 4/15 died in early morning. *Autopsy.* Much fat in the usual situations. Thymus large. Thorax negative. Liver and kidneys congested, spleen not much. Pancreas much congested. Stomach contained about 50 cc. of liquid with bile and blood in it; mucosa extremely hemorrhagic; no ulcers; two round worms. Duodenum contained bile, blood and

round worms; mucosa not congested, an ulcer at pyloric end. Jejunum, no congestion, no blood. Ileum, blood and bile in contents; a few congested patches in mucosa in upper part. Large bowel empty; mucosa congested, especially in rectum. Cecum empty and no congestion. Uterus early pregnancy, 5 embryos.

If fertilization in dog 1176 was due to the last coition it must have occurred within a very few days of the second operation, and perhaps could even have occurred subsequently to it. The protective influence of pregnancy then might not develop in time to permit a very prolonged survival.

In dog 1162 the time of coitus was known (18 days and 16 days before the second operation). She survived the loss of the second adrenal 13 days, 7½ hours. The embryos must therefore have been about mid term at the time of death of the mother. The onset of the fatal symptoms was more abrupt than usual, and it could hardly be said that a definite period of total anorexia preceded death. On the last day even she ate a little food. This is rare. It is impossible from the data to attribute death to any other cause than adrenal insufficiency, and it is difficult not to associate the extremely marked pathological changes in the gastro-intestinal mucosa with the rapid sinking of the animal. But why the intoxication so suddenly overcame the protection due to pregnancy is unknown. It may be that a toxemia, such as is sometimes associated with pregnancy, suddenly developed and reinforced the tendency to intoxication due to adrenal insufficiency hitherto held in check. Nothing heralded the change unless it were the moderate apparent rise in the non-protein nitrogen, on the morning of the last day. Had a blood sample been obtained later in the day it is probable that the rise would have been more pronounced. While the survival period is not longer than may rarely be found in control male or non-pregnant female dogs, it must be distinctly noted that a group of 5 dogs surviving 12¾ days, 13 days 7½ hours, 14 days 3½ hours, 14⅔ days and 15 days 2 hours respectively in a series comprising only 17 dogs already sharply differentiates table 1 of this paper from tables 1 and 2 of paper I.

Dog 1162. 12/22/25, right adrenal excised. Body weight 9.45 kgm. Weights on 3/10/26, 3/20, 3/26, 4/5, 4/17, 4/28 and 5/8 were 10.2, 10.7, 10.9, 11.0, 11.7, 11.6 and 11.5 kgm. respectively. On 4/10 and 4/12 mated with a stock male. On 4/27 blood taken for analysis.

4/28/26. Left adrenal removed at 2 p.m. She remained in excellent health and eating well up to 5/10, on which day it is noted that the appetite was not as ravenous as before, and that she was quieter. Blood (10 cc.) was taken for analysis from time to time as indicated in table 2. Her condition on 5/11 at 10 a.m., when blood sample was taken, is noted as very good, but at noon she only took a little of the meat and refused other food. The blood flow was slow. At 8 p.m., apathetic, had vomited twice (bile). Died at 9:35 p.m. Had a slight convulsion just before death. Half an hour earlier she vomited bile and had bloody diarrhea. *Autopsy* at once. Thyroid

normal. Thymus very large. Lungs, liver and kidneys normal. Spleen mottled. Moderately early pregnancy (7 pups in uterus). Stomach contained blood and bile; mucosa very hemorrhagic. Mucosa of entire gastro-intestinal tract was extensively and severely congested and hemorrhagic, and contained blood and bile. Pancreas very much congested.

Dog 996. 2/12/25, right adrenal excised. Body weight 8.0 kgm. On 3/3/26 excised left adrenal at 3 p.m. Body weight 8.7 kgm. On 3/4 not eating, vomited (acid). On 3/5 took only a little biscuit; inactive. On 3/6 quite well, ate biscuit and meat. 3/7 to 3/11, excellent appetite; quite normal in appearance and activity. On 3/12, vomited up milk (green with bile). Otherwise quite well; eating and active; very playful; weight 8.4 kgm. Up to and including 3/15 unchanged, very good health and appetite. 3/16, took little food; at 7 p.m. refused a pork chop bone (a delicacy). 11:30 p.m., vomited colorless acid liquid with mucus flakes.

3/17, 7:45 a.m. was seen in a wild dash across the room to the wall, yelling as if crazy; followed by convulsive side to side movements of head, which came on at intervals. Lay down exhausted. At 10:30 a.m. temperature 36.45°C; pulse 100; respiration 20. Body weight 8.8 kgm. Vomited (bile). At 4 p.m. semi-liquid stool, with much bile pigment and some spots of blood (confirmed by microscope). Took no food today.

3/18. At 12:10 a.m. pulse 106, respiration 22. Somnolent. 7 a.m. has vomited (bile). Quite apathetic. Looks ill and weak but can walk, although hind legs wobbly. Is not profoundly asthenic. At 10 a.m. pulse 108 (regular); respiration 24; temperature 36.56°C. At 1 p.m. pulse 58 (occasional double beat). Dog lying on side; can be roused. At 2:15 p.m., feces with some blood. Semi-conscious. Pulse 55, regular and not weak. At 3:45 p.m. comatose. Obtained blood from vein (she did not feel needle). Erythrocyte count of oxalated blood 8,500,000; leucocytes 32,000. Serum 39.7 cc. in 100 cc. of defibrinated blood. Conductivity of serum, $K\ (25°) \times 10^4 = 100.7$; and the same for serum from clot. At 4:30 p.m. temperature 35.92°; pulse 56 (regular); respiration 22. At 4:40 p.m. involuntary urination; some watery, bloody matter from rectum; pulse the same. Died at 4:50 p.m. *Autopsy* at once. Much blood in uterus (placental); 6 pups (7.5 cm. from root of tail to tip of nose). Wounds clean. Lungs normal. Spleen and liver, some congestion. Stomach contained 50 cc. of green liquid strongly acid to litmus; no blood; mucosa not congested. Duodenum, no congestion, except one or two hemorrhagic spots. Jejunum and ileum markedly congested and hemorrhagic (less congestion in lowest part of ileum), much blood in lumen. Colon much congested and contained blood. Cecum not congested but contained some blood clots. No worms anywhere; and none passed since operation. Some of the amniotic sacs contained clear liquid, others bloody liquid. On opening the abdomen the intestines looked very pale. The usual search revealed no accessory adrenals. The bladder contained some urine (sp. gr. 1,016).

The halving of the pulse rate, sometimes quite exactly, is not infrequently seen in dogs dying from adrenal deficiency, suggesting heart block. We have not noticed any difference between the pregnant and the control animals in this regard. Various irregularities may also be observed in the rhythm. Changes may occur within a very short time, the pulse reverting to its original rate and becoming regular, and again showing the abnormal rhythm perhaps many times in the last days, or in the case of dogs injected with Ringer-dextrose solution much earlier.

Dogs 1026 and 1002 were injected with Ringer-dextrose solution, at a

time when we did not know that pregnancy exerted a protective influence. Formally therefore it is impossible to say whether they owed their long survival, in both cases beyond anything seen among the controls (into the 18th day in 1026 and into the 33rd day in 1002) to the injections or to pregnancy, or to both. Perusal of the protocols seems to indicate that in 1026 parturition, perhaps excited by the second operation and beginning within less than 3 days of it, was preceded and accompanied by severe toxic symptoms which would probably have killed the animal had they not been successfully treated by the intravenous Ringer-dextrose injections. The animal was in very late pregnancy when the second adrenal was removed, and survived 14 days after delivery. Dog 1002 was not so near term, although in advanced pregnancy, and gave birth to her pups about 16 days after the second operation. Up to this time she was in very good health, and it is likely that this was due to the protective influence of the pregnant state, the injections perhaps having no effect. As stated, it is of course impossible to be absolutely sure of this. The matter is of no importance, as there are instances of longer survival among the pregnant dogs which were not injected and among the injected dogs which were not pregnant.

Dog 1026, in advanced pregnancy. 5/8/25, right adrenal excised. Body weight 11.5 kgm. On 6/16/25 removed left adrenal at 2:00 p.m., in 10 minutes. Body weight 13.3 kgm. Post-operative recovery excellent. At 10 p.m. in good condition.

On 6/17 at 7 a.m., lying on side, in bad condition. At 10 a.m. comatose; saliva dribbling from mouth. At 10:30 a.m. an intravenous injection of Ringer-dextrose solution caused slight improvement, but still semi-comatose; unable to raise head or use legs. Opened eyes, looked about and seemed to recognize bystanders, which she could not before the injection. At 11:20 a.m. got up and walked about the cage; fully conscious; passed semi-liquid feces (mucus and green bile); followed by a drop or two of red blood from vagina. Refused milk and water. At 2:30 p.m. drank fair quantity of water. 7 p.m. has defecated semi-liquid, yellow stool; has also vomited. Drank more water. At 11 p.m. same condition.

6/18, 7 a.m., semi-comatose, yelling from time to time. Another semi-liquid stool. At 7:30 a.m. Ringer-dextrose solution was injected. Afterwards she lapped some water and was much better. Before the injection no fetal movements were seen, but after 200 to 400 cc. had been injected, active movements were observed over the abdomen. Coma disappeared during the injection. At 4:30 p.m. has been comatose for ½ hour. Gave another injection, and coma disappeared. At 5 p.m. she was standing up; quite intelligent now and responds to petting. At 8 p.m. micturated. At 10 p.m. took water.

6/19, 12:30 a.m., unchanged. At 8:15 a.m. yelled and had a violent tetanic convulsion. Injected 1000 cc. Ringer with 20 grams dextrose. She walked from the treatment room to her bed and was much improved. Salivated and micturated. At 12:30 p.m. walked about for a while and dropped a pup; walked away from it. Condition fairly good all day. At 5:30 p.m. gave a few whines (labor pains?), then gave birth to another pup. About 15 minutes later, 2 more pups were born. She attended to them without assistance and licked them and herself in the usual manner. At 9 p.m. she had cleaned herself up well and was nursing 3 pups. At 9:30

p.m. injected 500 cc. Ringer with 10 grams dextrose. She urinated copiously, then took a run in the hall, came back to the pups and cleaned them thoroughly. She is better than at any time since the day after the operation.

6/20. Her condition is excellent. She is nursing 4 pups, and 4 are dead. At 11:30 a.m. ate a good meal (meat). At 3 p.m. injected 1000 cc. Ringer with 10 grams dextrose. She urinated copiously.

6/21. Condition of mother very good, but more pups dead. At 10:30 a.m. injected 1000 cc. Ringer with 10 grams dextrose; thereafter she ate meat.

6/22. The mother and 2 pups are in very good condition. In the morning she jumped over the cage to greet the observer. At 3 p.m. injected 850 cc. Ringer with 10 grams dextrose. She ate milk and bread, little meat. Defecated and urinated. In excellent condition. On 6/23 to 6/27 the injections were given daily. The mother remained in excellent health and the pups throve.

6/29. 9 a.m. She seemed a little less active. Ate a little biscuit. Received injection of 1000 cc. Ringer with 20 grams dextrose. Urinated copiously and defecated (solid, then liquid feces, blood-stained). At 11 a.m. refused food (biscuit and meat); urinated largely; passed some bloody mucus, then almost pure blood from rectum. At 1 p.m. vomited the biscuit eaten in the morning; no bile; reaction, acid. Urinated.

6/30. Ate a fragment of biscuit in the morning, nothing else all day; defecated several times in night (no blood). At 11:00 a.m. injected 1000 cc. Ringer with 20 grams dextrose. Vomited bile during the injection. Immediately after the injection passed semi-liquid stool with blood clots and some bloody mucus which came from the vagina. (Vagina has been bloody since parturition). Urinated and salivated considerably. Nursed pups.

7/1. Taking no food. At 11 a.m. injected 750 cc. Ringer with 20 grams dextrose. Semi-liquid stool immediately after with some blood spots in it. The dog is not asthenic.

7/2. This morning in walking she has some wobble. At 2 p.m. injected 1000 cc. Ringer with 20 grams dextrose. Vomited bile-stained mucus and passed liquid stool slightly blood-stained. Taking no food. Salivating.

7/3. She is getting weak. Taking no food and very little water. At 11 a.m. injected 850 cc. Ringer with 15 grams dextrose and 0.0001 gram adrenalin. After injection vomited mucous liquid (acid, no bile), urinated and defecated (liquid stool, no blood). Bloody mucous discharge from vagina. At 4 p.m. twitching and convulsion. Vomited bile. Died between 7 and 8 p.m. *Autopsy* at once. Lungs, liver and spleen normal. Kidneys moderately congested. Uterus congested and boggy. Stomach contains about 50 cc. of bile- and blood-containing liquid; mucosa hemorrhagic; 6 or 7 ulcers at the pylorus. Some gastric contents in esophagus. Duodenum contains 4 or 5 cc. of bloody liquid; mucosa congested. Contents of stomach and duodenum show weak oxyhemoglobin spectrum, but strong hemochromogen with KOH and $(NH_4)_2S$. The entire ileum and jejunum are hemorrhagic, the ileum more than the jejunum; about 25 cc. of bloody fluid (strong oxyhemoglobin spectrum). Colon and rectum to anus very hemorrhagic. Some bloody feces in colon, also in cecum. Ileo-colic valve hemorrhagic and pigmented.

Dog 1002. 2/24/25, excised right adrenal. Body weight 11.6 kgm. On 3/9/25 excised left adrenal at 2:30 p.m. Body weight 13.25 kgm. Perfect post-operative recovery. On and after 3/10/25 she received daily an intravenous injection of a liter of Ringer with usually 3 to 6 grams dextrose. The ordinary reactions to the injection were salivation, copious micturition and defecation (ultimately liquid). There was some vomiting of food but this was largely overcome by feeding her early in the morning. She remained in good health till 3/25, when 6 pups were born, one

dying at once. The others were nursed by the mother and well cared for. She took plenty of milk, but vomited some of it (curds and green bile). She ate well on 3/26 and vomited once or twice (green). From 3/27 to 4/2 she went on well, and the remaining pups throve, the mother having plenty of milk in her breasts. On 4/2 she vomited (green) and did not care much for food.

4/3. Body weight 10.35 kgm. At 8 a.m. she was seen to be wobbly on hind legs; less active and somewhat apathetic. At 8:45 a.m. she let out a series of the peculiar (epileptic-like) yells. At 11 a.m. apathetic (semi-comatose?). Has not urinated today, even after injection of 750 cc. Ringer with 6 grams dextrose. Has milk in breasts and pups are getting it. At 7 p.m. vomited 3 times; first vomit quite acid to litmus; second faintly acid; third (bile) neutral. Pulse 98 to 100, and regular. At 8:30 p.m. injected a liter of Ringer with 10 grams dextrose. After injection she walked without wobble, became less apathetic and cleaned the pups thoroughly.

4/4. 8 a.m. She voided considerable urine in the night. Ate a little biscuit. At 11:30 a.m. pulse 48 to 52 and irregular at times. Very weak and somnolent. At 11:45 a.m. injection of a liter of Ringer with 12 grams dextrose. She did not feel the needle; unconscious. Temperature 38.8°C; pulse 56; sighing respiration 16 to 20. With beginning of injection pulse became more irregular and slower, later faster (120) and regular. After injection voided much urine, walked well; became livelier, ate some food, nursed the pups and bathed them actively. Later vomited (bile and food). At 8 p.m. somnolent and apathetic; pulse 68, regular; very wobbly in walk. At 8:30 p.m. injected 750 cc. Ringer with 12 grams dextrose. Urinated well; the immediate benefit is striking. She walked without wobble; and gave much attention to the pups. After each injection as she brightens up she nurses and bathes the pups.

4/5. Seemed much improved. Ate some food; walked well; actively attending to the pups; growled at other dogs. At 11:30 a.m. got injection (a liter Ringer with 12 grams dextrose). Walked well. But at 8:30 p.m. she was unable to stand; voided much urine while lying on her side. At 9 p.m. injection of 750 cc. Ringer with 10 grams dextrose produced great improvement. She walked well, went to pups when they whined, and then walked about the room; micturated, etc.

On 4/6 her condition continued good. On 4/7 at 11 a.m. she cannot stand up; very weak. At 11:30 a.m. emitted the peculiar cry. Received injection of a liter Ringer with 15 grams dextrose. Urinated well; loose yellow stool; vomited (acid, no bile). 2 p.m., heart 120 to 130, rather weak. Neglected pups this afternoon for the first time. At 9 p.m. weak; pulse weak and rapid. Injected a liter Ringer with 20 grams dextrose. Urinated well.

4/8. Much better than last night. Vomited (acid, green). At 11 a.m. injected a liter Ringer with 2 grams dextrose. Much urine. Vomited (green). Unsteady on hind legs when walking. Taking no food. At 9 p.m. asthenic. Injected 800 cc. Ringer with 15 grams dextrose; immediate temporary improvement as usual.

4/9, 6 a.m. blood in stool. In morning walked well; nursed pups. At 10 a.m. injected a liter Ringer with 10 grams dextrose. Took no food today. Walked well, nursed pups. At 6 p.m. unchanged. Has not taken water for days. Injected a liter Ringer with 20 grams dextrose; usual results; went to pups and nursed them.

4/10, 7:45 a.m., urine is concentrated; gave an excellent reaction for bile pigment (Gmelin). This is rare. Cold HNO_3 test negative for coagulable protein. No sugar. 9 a.m. injected a liter Ringer with 10 grams dextrose; temporary benefit. At 5:30 p.m. comatose; did not feel needle when injection was made of a liter Ringer with 20 grams dextrose. After injection walked back to room but did not go near pups, which she abandoned today again. She did not urinate nearly as much as usual after the last injection.

4/11. Died about 7 a.m., without convulsions. Had vomited (neutral, bilious). *Autopsy* at once. Lungs normal, no fluid in pleural, and no excess of liquid in pericardial sac. Liver and spleen normal. Much liquid in peritoneal cavity. Pancreas somewhat congested. Stomach contained 80 cc. of clear yellowish green bile of rather low specific gravity compared with bile from gall bladder. No blood pigment. No special congestion in mucosa of stomach. Duodenum, some congestion of mucosa, contained bile with some blood pigment. Small intestine contains blackish material in flakes on surface of mucosa. They are blood clots. Mucosa of jejunum and ileum not specially congested. Large intestine some congestion, and some blood in contents; mucosa hemorrhagic especially in lower part down to anus. Small punctate hemorrhages in cecum. Kidneys congested. Glands under thymus abnormally large. Thyroid not enlarged. Urinary bladder empty. No worms found, nor were any passed during the time the dog was under observation.

With dogs 1192 and 1034 we reach a group such as has no parallel among the control animals. Of 17 pregnant animals, 7 lived into the 23rd day after the second operation, or longer. If the two injected dogs are left out, we have 6 animals in 15 which lived into the 23rd day or longer, namely 1192 into the 23rd day; 1143 into the 26th day; 1034 into the 27th day; 1036 into the 47th day; 1157 into the 58th day, and 1174 into the 59th day respectively. It may be recalled that among the 74 control male and non-pregnant female dogs only two survived as long as into the 15th day. This is ample proof of the protective influence of pregnancy after loss of the adrenals. Of the 6 animals, the three which survived longest were in early pregnancy at the time of removal of the second adrenal (1036, 1157 and 1174). Two of the others (1143 and 1192) were in late pregnancy; while one dog (1034) was about mid term.

Dog 1192. 2/12/26, right adrenal excised. Body weight 9.7 kgm. On 3/3 removed left adrenal at 11:15 a.m. Body weight 10.4 kgm. At 5:30 p.m. walking about in very good condition. Her condition remained excellent and appetite very good up to 3/16. Weight on 3/10, 10.6 kgm.

3/17 at 1:00 a.m. several pups have been delivered. At 9 a.m. nursing 4 pups; excellent condition. Up to 3/21 she remained in good health and likewise the pups, which were cared for and suckled in the normal manner. Body weight on 3/20, 10.0 kgm. She barks viciously at dogs approaching her cage.

3/22, 2:30 p.m., she is eating well, but lies on bed more than heretofore. On 3/23 at noon she has eaten little, if any, of her meal (meat). Otherwise seems alright. Ate the food later on. On 3/24 in the morning vomited (bile), and again at 3:30 p.m. Refused food all day. On 3/25 at 1:00 p.m. in poor condition. Refused all food. Took no notice of pups. Lying on bed. Quite apathetic. Had a convulsion and yelled a number of times during the day; vomited bile. At 5:30 p.m. semi-comatose and completely asthenic. At 10:45 p.m. found dead (probably dead for an hour or two). *Autopsy* next morning. Plenty of fat in usual situations. Thymus small. Liver congested; spleen moderately congested. Kidneys normal. Pancreas considerably congested. Stomach, marked congestion of mucosa, small ulcers about pylorus and pyloric end of fundus; contents bile. Duodenum, not congested; contained bile, large ulcer opposite pancreatic duct. Jejunum not congested; contained some bile-colored material. Ileum, two or three small hemorrhagic patches in mu-

cosa; contents small amount of bile and blood. Large intestine, no congestion, no blood in contents.

Dog 1034 gave birth to 5 pups, 23½ days after removal of the second adrenal. They were about full term, suckled well and were in all respects viable. The average period of gestation being 60 days, we must assume that at the time of the second operation, gestation was more than half over. For the first two days after parturition she was in very good condition, nursing the pups and eating. Then quite abruptly she began to go off, and in a few hours was dead. An injection of Ringer dextrose solution just before the end produced only slight temporary benefit. This is not the typical course of events in a dog dying of adrenal insufficiency; there is a longer period of decline after the serious symptoms have come on. It is difficult to resist the suggestion that in this and similar cases some complication connected with parturition is responsible for the quickly fatal termination.

Dog 1034. 5/28/25, 10:00 a.m. erythrocytes 6,450,000; leucocytes 18,500; Hb 94 per cent (by Haldane's scale). Blood sugar 0.087 per cent. Serum 59.2 cc. in 100 cc. blood (electrical method), 59.5 cc. by hematocrit. (The hematocrit reading for the serum should be somewhat less because a red color extends for a considerable distance between the clear serum and the meniscus of the sediment, increasing regularly in depth of tint from clear serum to sediment.) Conductivity of serum, K (25°) $\times 10^4$, 131.7. 6/1/25, 10:00 a.m. erythrocytes 6,000,000; leucocytes 16,800. Hb. 86 to 88. Blood sugar 0.093 per cent. 6/1/25, excised left adrenal. Body weight 8.3 kgm. On 6/8/25 in the afternoon, the erythrocyte count was 5,480,000; leucocytes 21,000; Hb 74; blood sugar 0.094 per cent; serum in 100 cc. blood, 67.3 cc. Conductivity of serum K (25°) $\times 10^4$, 127.8. Serum Ca 10.7 mgm. per 100 cc. 6/9/25, excised right adrenal at 2:45 p.m. Body weight 8.9 kgm.

6/13. Active and eating well. In forenoon erythrocyte count, 6,200,000; leucocytes 32,500 (stitch abscess on left side which is being drained and healing well); Hb 82 to 84. Blood sugar 0.12 per cent. Ca 12 mgm. in 100 cc. serum. Serum in 100 cc. blood 71 per cent. Conductivity of serum K (25°) $\times 10^4$, 125.6.

6/19/25. In forenoon erythrocyte count, 4,200,000; leucocytes 30,500; Hb 67. Ca in 100 cc. serum 12.5 mgm. Blood sugar 0.118 per cent. Serum in 100 cc. blood 74.9 cc. (very high for a dog). Conductivity of serum K (25°) $\times 10^4$, 129. 6.11 p.m. diarrhea. On 6/20 vomited bile, but otherwise alright.

6/23. In forenoon erythrocyte count 4,700,000; leucocytes 30,000; Hb 66. Blood sugar 0.045 per cent. Ca in 100 cc. serum 12.5 mgm. Serum in 100 cc. blood 66.9 cc. Conductivity of serum 142.4. The dog was not eating well today for the first time. Seems out of sorts, but is not weak. From 6/24 to 7/1 was again in very good condition, eating well; normal stools; no vomiting.

On 6/26, condition excellent. (In forenoon.) Erythrocyte count 3,950,000; leucocytes 29,500; Hb 50; blood sugar 0.11 per cent. Ca in 100 cc. serum 11.6 mgm. Serum 77.5 cc. in 100 cc. blood. Conductivity of serum K (25°) $\times 10^4$, 133.8. 7/2, did not eat meat but otherwise seems very well. On 7/3 in early morning 5 pups were born. On that day and 7/4 and 7/5 she was in very good condition, taking food, nursing and cleaning the pups.

On 7/6 at 8 a.m., the blood was found to be greatly concentrated. Erythrocyte

count 12,000,000; leucocytes 9,200; Hb 102; serum in 100 cc. blood 45.3 cc. Conductivity of serum 128.4. The blood sugar was diminished to 0.05 per cent. Ca in 100 cc. serum, 14 mgm. The dog was weak and semi-comatose. Injection (after obtaining blood sample) of 800 cc. Ringer with 10 grams dextrose (at 8:30 a.m.) produced only slight improvement, and she died at 9:15 a.m. *Autopsy* at once. Lungs filled with aspirated stomach contents. Liver and spleen engorged with blood. Enlarged lymph glands on both sides of bifurcation of aorta and cava. No residual or accessory adrenal tissue found. Pancreas pale, no congestion (this is unusual, perhaps connected with the injection of Ringer just before death). Uterus subinvoluted, contains some bloody liquid. Urinary bladder empty. Kidneys pale, peculiar greenish hue. Stomach contains frothy bile, no blood; no congestion. Duodenum contained many round worms, small amount of bile; no congestion; some blood in contents. Jejunum and ileum contained frothy bile with many worms; no congestion or hemorrhage of mucosa. Colon, hemorrhagic streaks in mucosa from ileo-colic valve to rectum. Both small intestine and colon contents gave good oxyhemoglobin spectrum; duodenal contents gave strong hemochromogen spectrum with KOH and $(NH_4)_2S$.

Dog 1036 was seen to have had coitus 5 to 8 days before the removal of the second adrenal, and this was the only opportunity for impregnation. She lived into the 47th day after the second operation and was in excellent health save for one or two mild temporary upsets, up to about 2 days before death. The immediate cause of death was perforation of a large duodenal ulcer into the peritoneal cavity. She was found in coma and collapse, but the diagnosis was not made in life. Ulcers were common in our series of adrenalectomized pregnant dogs in the stomach or duodenum, being found in 7 out of 16 cases, a much larger proportion than in the control males and non-pregnant females. But this is the only case in which perforation occurred. In another dog (1185) a duodenal ulcer was discovered *post mortem*, which had eroded all the coats, except the serosa. Dog 1036 was temporarily benefited by Ringer-dextrose injection, and parturition began. Several pups were born. They were viable but probably a few days to a week short of full term. How long the dog would have lived but for the accident of perforation cannot be known. But the history of dogs 1157 and 1174 suggests that she might at any rate have survived till gestation was completed or even longer.

Dog 1036. 6/2/25. Erythrocyte count 5,900,000; leucocytes 23,000; Hb 86; blood sugar 0.09 per cent. Serum in 100 cc. blood, 52.7 cc. K (25°) $\times$ 10^4 for serum 130.6.

6/3/25. Body weight 9.9 kgm. Left adrenal excised. 6/11/25, erythrocyte count 8,500,000; leucocytes 28,000; Hb 104; blood sugar 0.074 per cent. Ca, 11.5 mgm. in 100 cc. serum. Serum in 100 cc. blood 47.7 cc. K (25°) $\times$ 10^4 for serum 128.9. The blood specimens were always taken about the same time in the morning.

6/12/25. Body weight 9.5 kgm. Right adrenal excised at 2:00 p.m. Operation lasted 10 minutes.

6/15. Dog is always in the same good condition. Eating well. Erythrocyte count 7,250,000; leucocytes 24,000; Hb 110; blood sugar 0.08 per cent; Ca in 100 cc. serum 13.4 mgm. Serum in 100 cc. blood 42.8 cc. K (25°) $\times$ 10^4 for serum, 124.3.

6/19. The first wound is closed over; the second wound opened to drain, some sort of infection. Erythrocyte count 6,200,000; leucocytes 29,000; Hb 102; blood sugar 0.09 per cent; Ca in 100 cc. serum 14.3 mgm. Serum in 100 cc. blood 47.2 cc.; K (25°) $\times 10^4$ for serum 123.7. 6/23. Erythrocyte count 7,850,000; leucocytes 23,000; Hb 102; blood sugar 0.08 per cent; Ca in 100 cc. serum 16 mgm.; serum in 100 cc. blood 47.1 cc.

6/26. Erythrocyte count 8,500,000; leucocytes 30, 500; Hb 102; blood sugar 0.09 per cent; Ca in 100 cc. serum 13 mgm.; serum in 100 cc. blood 47.9 cc.; K (25°) $\times 10^4$ for serum 133.8. Condition excellent. She remained in very good health till 7/8, when it was noted that she was less lively and not eating as much as usual, has slight cough; otherwise seems alright.

7/9. Unchanged; ate some meat; stools normal. Erythrocyte count 8,500,000; leucocytes 24,000; Hb 100; blood sugar 0.09 per cent; Ca in 100 cc. serum 15.7 mgm.; serum in 100 cc. blood 43.5 cc.; K (25°) $\times 10^4$ for serum 131.7.

7/10 and 7/11. More active and eating well. Weight 9.15 kgm. On 7/15 she weighed 10.25 kgm. She continued in very good health up to 7/25, eating and acting normally. Abdomen is now quite big and breasts enlarged.

7/27. Vomited bile early in morning. Getting listless. Does not care now for petting; prefers to lie in a corner.

7/28, 8 a.m. Emitted a yell. At 8:20 a.m. she was comatose with head retracted and legs extended and very jerky. A slight touch on cage caused a clonic spasm. At 11 a.m. injected intravenously 750 cc. Ringer with 25 grams dextrose. After she had received 400 to 500 cc. she improved, raised her head and took notice of surroundings. Pulse became stronger, though still rapid. Near the end of the injection she began to strain and delivered a pup. The umbilical cord was tied off; but she could not attend to the pup. Though the coma was relieved by the injection she remained asthenic and lay on her side. From 2 to 4 p.m. she delivered 2 more pups (dead in sac when found). The first pup was trying to get milk from breasts but apparently unsuccessfully. Milk could not be squeezed from the nipples. She died about 5:30 p.m. Comatose for an hour before death; no convulsion or struggle. *Autopsy* at once. Thorax negative. Plenty of fat at usual sites. Omentum congested. Uterus contained 3 pups. Gall bladder contained semi-inspissated bile; its walls were somewhat thickened and adherent to duodenum. Pancreas much congested; liver and spleen only slightly congested. Kidneys, chronic inflammation; all abdominal lymph glands enlarged. Stomach, mucosa moderately congested; contained clear, viscid mucus, no blood. Duodenum, mucosa quite congested in lower half and moderately so in upper half; an ulcer about $\frac{1}{2}$ to $\frac{3}{4}$ inch in diameter opposite main pancreatic duct opening had perforated, and the wall was adherent to the gall bladder, with large lymph glands surrounding it. Duodenum contained brownish liquid (with blood pigment) and some of the same liquid was found in the abdominal cavity. Jejunum, moderate congestion above and marked in lower half; contents had bile and blood pigment. Ileum and colon moderately congested; blood pigment in contents. Rectum to anus much congested. Cecum and ileo-cecal valve not congested.

The longest periods of survival seen among pregnant dogs were in 1157 and 1174, which lived into the 58th and 59th day respectively, after removal of the second adrenal. These were also the longest periods of survival among all the dogs observed by us, the next longest being somewhat over $53\frac{1}{2}$ days in the case of a male dog (1170) treated by injections of Ringer-dextrose solution. Dog 1157 was not known to be pregnant

and there is no information in regard to the exact time of impregnation. The diagnosis of pregnancy was made in the absence of physical signs (she was a lanky animal and very thin in the loins) about a fortnight after removal of the second adrenal, because she had survived so long and continued in excellent health. She behaved just as she did before the operation, retaining all of her individual tricks and habits up to within a day before she died.

Those who believe that the rôle of the epinephrin discharge from the adrenals in physiological functions is important, particularly in the expression of the emotions, should study the actual behavior of animals not possessing adrenals during the period of good health. The same behavior can be seen for an indefinite time in cats or dogs with one adrenal excised, and the epinephrin output of the other interfered with or abolished by denervation or destruction of the medulla, or both. Total adrenalectomy may appear a less ambiguous experiment, but only to those who are not familiar with the subject. Minor digestive and other disturbances may occasionally develop in those pregnant animals which survive a long time. Dog 1157, for instance, was something of a glutton, and when the chance came, would gorge herself, and might then vomit semi-digested food (usually without bile), recovering after some hours or perhaps a day thereafter. Some such digestive upset may have been connected with the rise in blood sugar, to 0.18 per cent, for example, on July 9 and 17 (table 2). Parturition took place on the 48th day after the removal of the second adrenal. For two or three days thereafter she was somewhat less active but soon regained her usual behavior and continued in excellent health, nursing her pups, and eating well up to the last day of her life. It has already been mentioned, in discussing dog 1162, that we rarely see so short a period of anorexia preceding death. She died in the tenth day after delivery of her pups.

Dog 1157. 12/15/25, right adrenal excised. Body weight 8.7 kgm. Weights on 3/31, /26, 4/5, 4/17, 5/8, 5/19, 6/7, 6/10, 6/22, 6/29, 7/7, 7/19 and 7/21 were 11.7, 11.4, 11.3, 11.6, 11.9, 11.6, 11.9, 12.1, 12.45, 12.65, 11.4 and 11.9 kgm. respectively. On 12/18 to 12/21/25/ passed soft yellow stools with blood streaks. On 6/7 blood and urine analysis. On 6/10 excised left adrenal at 2:20 p.m. Very good post-operative recovery. Up to 6/19 she continued in excellent health, eating well, sometimes voraciously. Blood samples were taken on 6/12, 6/14, 6/16, 6/17, 6/18 and 6/19. On 6/20, vomited twice (small amounts, bile). Later vomited semi-digested food (without bile) several times. Otherwise seems in good health. Probably ate too much, taking leavings of other dogs. Blood sample taken in morning. 6/21. Quite well; appetite normal. She continued in a quite normal state as regards behavior and appetite. On 6/27 she ate little bread and milk but took a good meal of meat. On 6/28 unchanged. Blood sample obtained. On 6/29 ate biscuit and a good meal of rabbit. On 6/30 a blood sample was taken. Blood flow very good. Took a good meal of rabbit. She was in excellent health and continued so till 7/4, when she vomited all her food. She had been eating too much. She ate up part of the vomit. On 7/5 she

vomited food, but seemed otherwise in very good health, playful and very keen for food. On 7/6 she ate well (bread and broth but mainly rabbit). On 7/7 a blood sample was obtained (5 cc.). The dog was in splendid condition. On 7/8, ate meat, bread and broth, and rabbit. On 7/9 a blood sample got (5 cc.). In the afternoon vomited semi-digested food but seemed happy. She continued in excellent health, with good appetite, up to 7/26, when she seemed a little less alert and was not very anxious for food, although she took her meal later in the day than usual. On 7/27 she had some discharge (mucus) from vagina. On 7/28, at 6:00 a.m. three pups were born (one dead). The two living pups were at breasts but the mother seemed quite apathetic. She took no food at noon, but later took some milk. Coughed all day, and passed brownish loose stool in the afternoon and again in the evening. 7/29 and 7/30 she was gradually getting more active but she coughed a good deal and had diarrhea; appetite better. From 7/31 to 8/6 she continued in excellent condition eating well, caring for her pups and behaving like a normal dog. Blood samples (5 cc.) were obtained as shown in table 2.

On the morning of 8/6 she again began to cough badly and looked less energetic but was not at all asthenic. At 9:00 a.m. she ate a biscuit and about an hour later took a pan full of milk. She refused her regular meal of meat at noon and seemed to be getting more apathetic. There has been a (shreddy) discharge from the vagina since this morning. At 2:00 p.m. she refused milk, meat, or biscuit and from then till 5:00 p.m. she vomited four times. The first vomit was acid to litmus, later (more copious) was neutral and contained bile. Passed dark colored solid, later tarry, stool. At 9:30 p.m. she was quite asthenic and when placed upon her feet she was scarcely able to stand. Refused all food (a piece of duck with bone was also offered) and was very listless. Pulse 110, regular; temperature 37.8°C; respiration 18 to 22; blood flow very slow when sample was taken. She died some time between 8/6 midnight and the morning of 8/7. *Autopsy* at 7:00 a.m. Large amount of abdominal and omental fat. Right lung normal, left lung moderately congested. Thyroid slightly enlarged; thymus large. Liver congested; spleen and kidneys not congested. Stomach contained about 70 cc. bile-colored liquid; the mucosa of the fundus was hemorrhagic over an area of about one and one-half inches; few small erosions near pylorus but no ulcers. Duodenum contained bile-colored material; mucosa, no congestion whatever; the jejunum and ileum contained some mucus (bile-stained), a number of round worms and lower down a small amount of bile-stained fecal matter; only a few small hemorrhagic spots were found (in the lower part of the ileum), otherwise the small intestine was free from congestion. The colon was hemorrhagic throughout and contained some bloody and bile-stained fecal matter. The pancreas was only very slightly congested, probably not much more than was due to post-mortem hypostasis. The uterus was thick and contained the same material that was being discharged from the vagina for a day preceding death.

Dog 1174, the longest survivor among our series of pregnant dogs, was mated with a stock dog (1200) eight days before removal of the second adrenal. That was the only opportunity for impregnation. Parturition began fifty-five days after the second operation. She recovered well but only lived three days longer. Blood analyses (non-protein N, etc.) were made from time to time. These will be alluded to later.

Dog 1174. 1/7/26, right adrenal removed. Body weight 6.2 kgm. Weights on 3/10, 3/20, 3/26, 4/5, 4/17, 5/7, 5/17, 5/26, 6/7, 6/22, 6/29 were 7.3, 7.7, 8.0, 8.0, 8.5, 8.7, 8.3, 8.3, 8.3, 8.7 and 8.15 kgm. respectively. 4/9/26, she was successfully mated with

dog 1200. On 5/6 blood sample taken. On 5/7 left adrenal excised at 11 a.m. Very good post-operative recovery. Blood samples (10 cc.) were taken on 5/11, 5/14,5/20, 6/8 and 6/14, always in the morning (8 to 9 a.m.). The dog remained in excellent health all this time; she ate well; none of her habits were altered. For example, before the operations she was a great sleeper, and so she was after the operations.

On 6/14 she ate very little of her noon meal (meat) but took some ham (a delicacy) and some warm freshly cooked meat, But she did not bark at her food as was her habit. No diarrhea. Took much water, and at 10 p.m. seemed all right.

6/15. In perfectly good health and continued so, eating well and barking at her food, till 6/27, when she only ate half the usual amount of meat, but did not want bread and milk.

6/28. Blood taken for analysis. Ate some fresh lean meat, but is more dainty. As usual drinks much water.

6/29. Ate rabbit readily; growled as usual while eating. Refused biscuit but barked loudly at a neighbor for trying to get it. Very good condition.

6/30. Blood flow good during collection of sample. Ate a good noon meal (rabbit). Wants petting.

7/1. At 6 a.m. came out of cage at once and walked around room. At 8:30 a.m., a pup was delivered. The mother resents greatly the presence of another dog in the room, although previously quite friendly. Between 9 and 10 a.m., 2 more pups were born living. Nursed and cleaned in ordinary way by the mother, who ate the placentas. Another pup came at 2 p.m. The mother attended to them all in the normal manner. At 9 p.m. a blood sample was taken; the flow was very good. The dog now ate a good meal of rabbit, growling and barking as usual, and chewing up the bones then went to sleep.

7/2. 7 a.m. Good condition. 8 a.m. blood (5 cc.) taken; good flow. Taking care of pups, coming out of cage from time to time to micturate, etc. Ate very little all day (only a morsel of rabbit and one or two bits of bread soaked in rabbit broth). Otherwise she seemed well; and behaved as usual. Nurses pups. 7/3. Apparently unchanged, but ate no food all day. The pups are all dead (overlain?). She had neglected them all day. Walks quite well. Drinks plenty of water. At 10 a.m., torpid; looks unhappy; surface cold. Ate nothing today. At 11:15 a.m. blood sample got (poor flow). She did not feel the needle. Found dead at 3 p.m. (warm, no rigor). *Autopsy* at once. Lungs normal. Right heart not as dilated as usual. In right auricle and ventricle are thrombi, red and white; white thrombi in veins near heart. Thymus large. Thyroid nothing special. No liquid in pleural or pericardial sacs. Plenty of fat everywhere. Milk easily pressed from teats. On opening the abdomen all the organs except liver and pancreas seem pale. Liver considerably, and pancreas moderately congested (++). Omentum pale; much fat. Kidneys anemic rather than congested. Spleen not congested. Gall bladder full of bile. Urinary bladder empty. Intestines appear pale and the lower two-thirds of small intestine much contracted. Stomach contains yellowish bile, no blood; mucosa pale, not congested. No ulcers or erosions. Duodenum not congested, contains bile. Jejunum in upper part contains some blood spots, and bile everywhere, mucosa but little congested. Lower part of jejunum and all ileum and large intestine free from congestion; no blood in lumen. No worms anywhere. Sites of operations showed no adrenal remnants. No accessories. The unusual dryness of the field in making the autopsy was noteworthy. Is this connected with loss of blood and other losses of fluid in recent parturition?

Blood examinations. Blood samples were analysed from time to time in a number of the cases, as in the controls. Table 2 gives the data in

the four dogs most completely examined. The non-protein nitrogen, urea nitrogen, uric acid, creatinin (preformed and total), amino-acid nitrogen, undetermined fraction of the non-protein nitrogen and chlorine (expressed as NaCl) are given as milligrams in 100 cc. blood. Dextrose is given as grams per 100 cc. In dog 1157, there was no significant change before parturition, in any of the constituents examined, except that twice the blood sugar went to a distinctly hyperglycemic level (0.18 per cent). This was associated with a temporary digestive upset. On the second occasion (7/17) the chlorine was also decidedly increased. Following parturition the non-protein and urea nitrogen began to mount, with a drop to nearly normal on the third day, rising again next day. In dog 1162, which died when gestation was about half completed, the non-protein and urea nitrogen showed no significant change. Even on the morning of the last day of life the values were no greater than previously seen in this dog, although the appetite was already obviously diminished and the blood flow was slow. The fatal symptoms developed very rapidly and she died in about twelve hours after the last blood sample was taken. It is probable that if a blood specimen had been obtained nearer the end, a decided increase would have been seen, as usual, in the non-protein and urea nitrogen.

In dog 1174 definite changes were observed on 6/30, the day before parturition began. The non-protein nitrogen, urea nitrogen and the undetermined fraction all mounted. The blood sugar came down to 0.06 per cent and the chlorine increased distinctly. Next day, while the pups were being born, the non-protein and urea nitrogen came back to normal; the sugar rose to 0.08 per cent, while the chlorine remained rather high. On 7/2, when she had virtually stopped eating, the non-protein nitrogen and urea nitrogen began to go up again. On 7/3, when she refused all food, but nursed and cared for the pups the rise continued and the chlorine reached the highest value seen in this animal. This is a contrast to the control adrenalectomized males and non-pregnant females in whose blood chlorine tends to diminish with the rise in non-protein and urea nitrogen. The blood sugar sank again to 0.06 per cent. On 7/4 the last day of life the non-protein and urea nitrogen and the undetermined fraction underwent a further great increase, but the sugar went back to normal (0.08 per cent).

In dog 1198, which survived the second operation less than a week, it is an interesting point that the non-protein and urea nitrogen were already decidedly increased on 5/9, two days after the second operation. Her condition was not first class, and she never regained much appetite. On 5/12 and 5/13 (12 hours before death) the non-protein and urea nitrogen and the undetermined fraction went on rising rapidly. The chlorine diminished distinctly as the non-protein nitrogen increased. This was seen on the first date (5/9) on which an increase in nitrogen was shown

and thenceforth continued, the maximum diminution being 20 per cent. This animal resembles in all respects the control male and non-pregnant adrenalectomized dogs. Everything suggests that adrenal insufficiency was the dominant factor in the rapid onset of the symptoms and in the fatal result. The unusual severity of the intoxication due to this cause completely neutralized any protective influence of pregnancy. There are facts which suggest that generally late pregnancy (as in this dog) affords a smaller degree of protection than early pregnancy; although there are not enough data to enable a definite conclusion to be drawn.

On the whole, no striking differences appear in these data from pregnant dogs and those already discussed in paper II from control males and non-pregnant females, unless it be the increase in non-protein and urea nitrogen (with some drop in blood sugar) preceding parturition in dog 1174, and the return to normal after parturition has set in. We do not feel capable of answering the question whether the increase is associated with a breakdown of the protection afforded by pregnancy against adrenal insufficiency, quickly compensated by changes connected with parturition, or is a change associated entirely with the approaching labor. As regards the increase in non-protein and urea nitrogen and in the undetermined fraction in the last two days of life, there seems no reason to doubt that it is connected in some way with the intoxication which at the last overwhelmed the animal, as it does the male and non-pregnant adrenalectomized dogs, and in which the derangement due to the absence of the adrenals is at least the principal factor. Whether in the parturient animal other factors not directly connected with adrenal insufficiency may be superadded cannot be determined from the small number of data at our disposal. It would, however, appear probable.

Discussion. As regards the factors involved in the protective influence of the pregnant state, it is impossible to say anything very definite at present. The only point which appears certain is that protective influence is still exerted when the uterus has been emptied by a normal delivery. The protection is therefore not simply due to the adrenals of the embryos functioning for the mother also. This is seen most clearly in dog 1143, which lived into the 26th day after delivery of 6 pups. They were viable, had hair, and were either at full term or not far from it. Parturition, however, was certainly induced by the second operation as it began within half an hour of completion of the operation. The mother, as is always the case with adrenalectomized pregnant dogs, unless they themselves are ill, behaved exactly like a normal dog in parturition, ate the membranes, cleaned and nursed the pups and showed all the usual maternal instincts. She certainly was not being kept alive by the adrenals of the embryos, as she lived far longer after delivery than any of our control adrenalectomized dogs. Further, among the most striking in-

stances of prolongation of life in our series of pregnant animals were cases where impregnation must have occurred not more than a week (perhaps considerably less) before the second operation. The adrenals could therefore have been but little developed when the critical stage arrived, perhaps a week or even less after the removal of the second gland. It is possible, however, that the cortex may have been to some extent capable of function. Nothing is definitely known in regard to this. Epinephrin has been found in the developing adrenals of the ox, hog and sheep at a very early stage, 6 to 12 weeks after conception in the ox, i.e., one-sixth to one-third of the gestation period (Fenger, 1912). McCord (1915) obtained reactions characteristic of epinephrin from extracts of bovine fetal adrenals during all developmental stages at which macroscopic recognition of the glands is possible (from the sixth week on). Although the cortex and not the medulla is the important tissue for the matter under discussion, the precocious development of the medulla may indicate that the cortex also develops early to the functional stage. The survival of dog 1143 being of considerable importance for the question under discussion, some extracts from the protocol will be given. It is to be expected that further data could be obtained by artificially evacuating the uterus, as by Caesarean section. Nothing can better illustrate the rapid and complete post-operative recovery which we see habitually after adrenalectomy, than the fact that this dog, half an hour after the second operation, behaved precisely like a normal dog in regard to all the incidents of parturition and the care of the pups.

Dog 1143. 10/20/25 and 10/21, injected intravenously each of these two days a liter Ringer, with 10 grams dextrose, to test a sample of Ringer's solution. Thereafter no more injections. Vomited bile the first day. On 10/27/25 removed right adrenal. Body weight 10.4 kgm.

11/19, excised left adrenal at 10:45 a.m. Body weight 11.2 kgm. After an excellent post-operative recovery, parturition began and about 11:15 a.m. 2 pups were born. At noon another pup was delivered, to which the mother attended normally, ate the membranes, cleaned the pup and cuddled it. By 4 p.m. 3 more pups were born and were taken care of and nursed by the mother. They had hair, and were not far from full term. She continued in good health, eating well and nursing her pups (reduced to 4) till 11/24 when for the first time she refused her meal and kept away from the pups. On 11/25 she was all right again, eating well and taking care of the pups. On 11/26 unchanged. On 11/27 seems in very good condition, except that when walking the hind legs wobble slightly. The situation remained the same till 12/3, except that the wobble was now entirely gone. Her appetite was excellent; her behavior to the pups normal. She snapped at dogs coming too near her pups. She walked well and enjoyed a race in the hall. On 12/4, everything practically the same, except that the hind legs wobbled slightly as she raced along the corridor. Interested in rabbits. The pups' eyes are open, and they are doing well.

On 12/5 to 12/7, she continued to eat well and to attend to the pups. She looks like a normal dog, but is possibly more easily fatigued, although fond of running in the corridor. The slight unsteadiness on hind legs does not seem to bother her

much. She "went for" other dogs, chasing them away. On 12/7 one of the pups discovered and finished a dish of milk belonging to the mother. On 12/8 no wobble, but when she turned in walking or running there was a tendency to sway. On 12/9 to 12/13 her condition was very good. She ate well. She had lost some weight (9.05 kgm. on 12/9 and 8.35 kgm. on 12/14).

On 12/14 at 1:00 a.m. she was asleep with the pups on her bed. At 7 a.m. she was having fits. At 9 a.m. lying on her side, incapable of rising; in very poor condition. At 9:45 a.m., temperature 37.10. Pulse 88; respiration 24 to 26 a minute. Obtained a blood sample. At 10 a.m. a violent tetanic spasm lasting about a minute. She remained comatose all the morning. A tetanic convulsion once in 20 minutes, and later more frequently. Died at 4 p.m. *Autopsy* at once. Yellowish mucoserous discharge from vagina. Bile-stained fluid running from mouth. Plenty of milk in breasts. Thyroids small and pale. Not much subcutaneous fat; good amount of intra-abdominal, including mesenteric and omental fat. Thorax negative. Thymus very small. Pancreas considerably congested. Stomach contains small amount of bile-stained froth; no blood in contents. Small ulcers (3 or 4) in mucosa (2 to 4 mm. in diameter). Duodenum contains small amount of brownish material (blood pigment); large ulcer at pyloric end (over 13 mm. in diameter) extending down to peritoneal coat but not perforated. Some congestion in region of ulcer. Jejunum-ileum, hemorrhagic areas throughout portions of lower half; some blood pigment in contents. Large intestine hemorrhagic on ridges, no contents. Uterus large and boggy. Ovaries large, and corpora lutea prominent. Careful search made for accessory adrenals in the usual places but none found. No worms in alimentary tract. Kidneys congested; capsules strip less easily than normal. Spleen normal. Liver considerably congested.

As further evidence, although indirect, that it is not the adrenals of the embryos, or not these solely, which constitute the protective mechanism against adrenal insufficiency in pregnant dogs may be mentioned the fact that the adrenals of the pups do not appear to be enlarged as might be expected if they were making up by increased activity for the loss of adrenal function in the mother. Weighing of a considerable number of fetal adrenals would be necessary to determine the point exactly. That the embryos develop normally in the absence of the maternal adrenals may be considered as established. The theory that the adrenal cortex manufactures lipoids for the other cells, and especially the myelin of the nervous system has so little foundation in the way of established facts that it is curious it should be found "attractive" by some writers. Undoubtedly the tissues of the embryos get all the lipoids they need, and their nervous systems all the myelin required, in the absence of the maternal cortex. However it is not known whether in normal pregnant animals there is any period of gestation, even the earliest, during which an influence is exerted on the fetal metabolism by the mother's adrenals.

The significance of the adrenal hypertrophy which is described as occurring in pregnancy (Guiyesse, 1899) and still more in lactation (Verdozzi) has been alluded to by one of us (Stewart, 1921) in a previous paper. There it was pointed out how confused a picture is obtained if

all the statements in the literature as to the conditions which cause hypertrophy of the adrenals are accepted, and the assumption, apparently considered axiomatic by many writers, that hypertrophy is due to a call for increased functional activity is always granted. The fact is that many of the cases of alleged hypertrophied adrenals cannot be explained upon this basis. Even the so-called compensatory hypertrophy and hyperplasia of one adrenal when the other is removed is puzzling enough. For one adrenal cortex is far more than is sufficient to maintain health and life indefinitely. And the dog, which apparently seldom, if ever, has accessory adrenals and therefore might be expected on this theory to show a greater tendency to compensatory hypertrophy of the remaining adrenal than the rabbit, in which they are common, shows in fact relatively little tendency to it.

Among the maternal organs possibly concerned in making up in some way for the loss of function of the adrenals the corpora lutea might be thought of. Microscopically they have been supposed to resemble adrenal cortex. According to various writers (Goormaghtigh, 1925) the maximum physiological activity of the corpus luteum in the bitch is attained between the 10th and the 15th day after coition. It is true the supposed function studied had no relation to any possible vicarious substitution for the function of the adrenal cortex, and the criteria of increased function were simply increased intensity of change in the lipoid and fatty inclusions as followed by the microscope. Nevertheless if the corpus luteum is capable of contributing something to make up for the loss of the adrenals in the pregnant animal, the period of the 10th to 15th day would fit in well with the time at which in our animals a neutralizing or protective influence would certainly be needed, and in the case of the dogs whose time of mating was known, must have been exercised. If the corpus luteum is a factor in the protective influence of pregnancy it may be by its action on other organs such as the uterus, the other ovarian tissues, or even the mammary gland. Its best proved function seems to be the prevention of ovulation when pregnancy has occurred (Loeb and Hesselberg, 1917; Kennedy, 1925). Whether through the corpus luteum or independently of it the ovary, either the follicular or the interstitial tissue, or any of the other maternal organs mentioned may take a share in the protective influence. Some aid in the analysis of the problem may be obtained by removing one or other of the structures at different times after fertilisation. But up to the present no data are available which render it worth while to speculate. The same is perhaps still more evident in the case of the hypothesis that it is not any particular organ but some general or special change in the maternal metabolism, or the development of the fetal metabolism which enables toxic substances produced or retained in the absence of

the adrenals to be excreted, neutralized, destroyed or even utilized, or which, on the other hand, enables indispensable substances normally manufactured in the adrenal cortex to be supplied by some other organ, or even to appear as the results of other chemical sequences. The answer to some of the questions may be facilitated by comparative observations on different kinds of animals. In any case it would be of interest to determine the period for which the protective influence lasts in mammals having a much longer period of gestation than the dog. It is to be expected that it would be more prolonged. In a bitch the second adrenal was removed between the 3rd and 4th week after the beginning of the prooestrum. The possibility of coition was entirely excluded. She lived into the 37th day after the second operation. Her health remained good until a few days before death. Another bitch, found at autopsy not to be pregnant, had the second adrenal removed shortly before the end of oestrus. She lived just 32 days. Since nothing like this period of survival was seen in the 74 control animals, the suggestion is that the changes connected with prooestrum and oestrus exert an influence in neutralising for a time the effects of adrenal insufficiency. If this is the case, the influence of the oestrous cycle may be concerned in the protective effect of pregnancy. The obvious question, whether in the male an influence on the survival period can be obtained, e.g., by procedures calculated to cause hyperplasia of the interstitial cells of the testis, or even by restricting or encouraging the functional activity of the spermatogenous tissue, can only be answered by experiment.

SUMMARY

Pregnancy has a marked influence in dogs in prolonging the period of survival, in good health, after removal of the adrenals. Of 17 pregnant adrenalectomized dogs one survived for a period equal to the average gestation period (into the 59th day). One lived into the 58th day; one into the 47th day; one into the 33rd day; one into the 27th day; one into the 26th day; one into the 23rd day. Six of the others survived much longer (13th to 18th day) than the average of the control male and non-pregnant females. One of the dogs lived into the 26th day, after giving birth to a litter of pups immediately after the second adrenal operation. As no such period of survival or anything approaching it was seen among the 74 control dogs, it must be assumed that this animal was not being kept alive by the adrenals of the embryos.

Estimations of the blood non-protein and urea nitrogen, uric acid, creatinine (preformed and total), amino-acid nitrogen, "undetermined fraction" of the non-protein nitrogen, chlorine (expressed as NaCl) and dextrose were made on some of the dogs from time to time. An increase in the non-protein and urea nitrogen was seen, in one case the day before parturition. When the serious symptoms preceding death appear a

marked rise occurs in the non-protein and urea nitrogen and in the undetermined fraction. In this regard there is no essential difference between the pregnant and the control dogs, excepting dog 1157, which lived into the 58th day (10th day after delivery), and showed a high nitrogen value from the day after parturition till death, although she was in excellent health up to the day she died.

The so-called concentration of the blood (diminished proportion of plasma or serum relatively to corpuscles) was observed in the terminal stages in some of the pregnant dogs, as in the control dogs (Stewart, 1926).

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